QH611.L78.

THE DECENNIAL PUBLICATIONS OF
THE UNIVERSITY OF CHICAGO

THE RÔLE OF DIFFUSION AND OSMOTIC PRESSURE IN PLANTS

LIVINGSTON

QH611. L78.

Austin College Library.
Case. Shelf.
Number

Austin College Library.
Case..... .....Shelf.....
Number..... .....

THE DECENNIAL PUBLICATIONS OF
THE UNIVERSITY OF CHICAGO

THE DECENNIAL PUBLICATIONS

ISSUED IN COMMEMORATION OF THE COMPLETION OF THE FIRST TEN YEARS OF THE UNIVERSITY'S EXISTENCE

AUTHORIZED BY THE BOARD OF TRUSTEES ON THE RECOMMENDATION OF THE PRESIDENT AND SENATE

EDITED BY A COMMITTEE APPOINTED BY THE SENATE

EDWARD CAPPS

STARR WILLARD CUTTING ROLLIN D. SALISBURY

JAMES ROWLAND ANGELL WILLIAM I. THOMAS SHAILER MATHEWS

CARL DARLING BUCK FREDERIC IVES CARPENTER OSKAR BOLZA

JULIUS STIEGLITZ JACQUES LOEB

THESE VOLUMES ARE DEDICATED

TO THE MEN AND WOMEN
OF OUR TIME AND COUNTRY WHO BY WISE AND GENEROUS GIVING
HAVE ENCOURAGED THE SEARCH AFTER TRUTH
IN ALL DEPARTMENTS OF KNOWLEDGE

DIFFUSION AND OSMOTIC PRESSURE

THE RÔLE OF DIFFUSION AND OSMOTIC PRESSURE IN PLANTS

BY

BURTON EDWARD LIVINGSTON

OF THE DEPARTMENT OF BOTANY

THE DECENNIAL PUBLICATIONS
SECOND SERIES VOLUME VIII

9803

CHICAGO
THE UNIVERSITY OF CHICAGO PRESS
1903

Copyright 1903
BY THE UNIVERSITY OF CHICAGO

PREFACE

With the ever-increasing tendency to regard an organism as a complex of physical and chemical processes which may one day be analyzed and understood, there has necessarily gone hand in hand a tendency toward more and more accurate and quantitative investigation of the physics and chemistry of the cell itself. Among the various groups of physical and chemical phenomena that have been found to play important rôles in the life-process, and which, therefore, have been interrogated for answers to physiological questions, none has stood out within the past few years as more fundamentally important than those connected with diffusion and osmotic pressure. This field has thus far only been touched upon, and it would seem, judging from researches which have recently appeared, that the best and most far-reaching work therein is probably yet to come.

The present volume will deal with the past and present of diffusion and osmotic pressure from the standpoint of plant physiology. It has a double *raison d'être*. First, it was felt that there was need of some direct and not too exhaustive account of the essential physical facts and theories of the subject. The interest of the physical chemist here has lain mainly in the light which these phenomena have been able to throw upon the ultimate nature of matter and upon electrolytic processes. It has thus been difficult for the student of physiology who is not at the same time well versed in physical chemistry to obtain the information required for the prosecution of work in this field. Secondly, it seemed desirable to bring together in a general review the literature of this subject in its biological aspects, so that the promising and unpromising points for future research might become

9830

more apparent. The volume will thus naturally fall into two Parts, the first dealing with the purely physical aspect of these phenomena, and the second attempting to present in a more or less unified whole the physiological results which have so far appeared in this connection, together with their bearing upon each other and upon the vital problem as a whole. Chapter IV of Part II was presented to the Faculty of the Ogden Graduate School of Science of the University of Chicago in candidacy for the doctor's degree in 1901.

The author wishes here to express his thanks to Professor C. R. Barnes, of this laboratory, and to Professor Jacques Loeb, of the Hull Physiological Laboratory, for much valuable aid. Professor Barnes has kindly read the manuscript and has made many suggestions. The author alone is, however, responsible for whatever new departures are to be found in the book.

B. E. L.

THE HULL BOTANICAL LABORATORY,
The University of Chicago,
October 1, 1902.

TABLE OF CONTENTS

PREFACE - - - - - - - - - - - - - ix

PART I. PHYSICAL CONSIDERATIONS

INTRODUCTION - - - - - - - - - - - - 1

CHAPTER I. Matter and Its States - - - - - - - 3

I. Fundamental Theories of the Nature of Matter.
a) The Atomic Theory.
b) The Kinetic Theory.
II. The Three States of Matter.

CHAPTER II. Diffusion and Diffusion Tension - - - - 9

I. Gases.
a) Simple Gases.
b) Mixed Gases.
II. Liquids.
a) Simple Liquids.
b) Mixed Liquids.
III. Solids.
a) Simple Solids.
b) Diffusion of Two Solids.

CHAPTER III. Liquid Solutions - - - - - - - 16

I. Liquids in Liquids.
II. Gases in Liquids.
III. Solids in Liquids.
IV. Terminology for Solutions of Differing Concentration.

CHAPTER IV. Ionization - - - - - - - - - - 23

I. Of Gases.
II. Of Solutes in Liquid Solutions.

CHAPTER V. Osmotic Phenomena - - - - - - - 25

I. Osmotic Pressure of the Solute.
a) Non-electrolytes.
b) Electrolytes.
c) Colloids.
d) Osmotic Pressure in General.

II. Diffusion Tension of the Solvent.
III. Experimental Demonstration of Osmotic Pressure.

CHAPTER VI. Measurement and Calculation of Osmotic Pressure - - - - - - - - - - - - 35
I. Measurement of Osmotic Pressure.
a) Direct Method.
b) Indirect Methods.
1. Freezing-point.
2. Boiling-point.
3. Vapor Tension.
II. Calculation of Osmotic Pressure.
a) Non-electrolytes.
b) Electrolytes.

PART II. PHYSIOLOGICAL CONSIDERATIONS

INTRODUCTION - - - - - - - - - - - - 47

CHAPTER I. Turgidity - - - - - - - - - - 49
I. Protoplasm and its Limiting Membranes.
II. Plasmolysis.
III. The Permeability of the Protoplasmic Layers.
a) Test by the Plasmolytic Method.
b) Direct Test of Penetrability.
c) Absorption Test.
d) Test by Toxicity.
e) Test by Accumulation.
f) Test by Metabolic Processes.
g) Outward Permeability.
h) Variations in Permeability.
IV. Action of the Protoplasmic Membrane.
a) The Filter Theory.
b) The Solution Theory.
c) The Chemical Theory.
V. The Nature of the Osmotically Active Solutes.
VI. The Maintenance of Turgidity in Spite of Permeability to Certain Solutes.
VII. The Relation of Turgidity to Vital Activity.
a) The Retention of Form.
b) Mechanical Support.
c) Growth.
d) Curvature.
e) Work.
VIII. Summary of the Chapter.

CHAPTER II. Absorption and Transmission of Water - - 91

I. Absorption of Water.
II. Transmission of Water.
a) Water Loss.
(1) Evaporation.
(2) Water Pores and Nectaries.
(3) Exudation.
(4) Summary of Water Loss.
b) Upward Movement of Water in Trees and Other Tall Plants.
III. Summary of the Chapter.

CHAPTER III. Absorption and Transmission of Solutes - - 115

I. Absorption of Gases.
II. Absorption of Dissolved Solids and Liquids.
III. Transmission of Solutes.

CHAPTER IV. Influence of the Osmotic Pressure of the Surrounding Medium upon Organisms - - - - - 124

I. Introductory.
II. Presentation of Material.
a) Influence upon Growth and Form.
b) Influence upon Reproduction.
c) Influence upon Irritability.
(1) Changes of Irritability.
(2) Osmotaxis.
d) Analogy between the Effects of High Osmotic Pressure of the Medium and Those Produced by Other Water-Extracting Processes.
III. Summary of the Chapter.

INDEX - - - - - - - - - - - 145

PART I

PHYSICAL CONSIDERATIONS

INTRODUCTION

In the following treatment of the physical phenomena of diffusion and osmotic pressure no attempt is made to be exhaustive. Certain aspects only of the present conceptions of these matters among most physicists and chemists[1] are discussed, and every discussion is presented with the sole aim of clearing the way for the physiological discussions which are to follow. Thus, for example, the whole subject of atomic and molecular weights and their experimental determination — so important to the chemist, but not primarily interesting to the physiologist — is entirely omitted. Also it may be added that no attention is given to a historical treatment of this part of the subject, the excellent chemical treatises which are now available rendering this unnecessary.

In the present Part very little is original with the author, excepting the mode of presentation. The various texts and the original papers have been drawn upon wherever it has seemed expedient. Footnotes give the names of the authors

[1] A general confusion among younger students with regard to the way in which these conceptions take the form of theories makes it seem expedient to call attention to the following points: A scientific theory does not pretend to state the truth. It may sometimes state a part of the truth, but this is not primarily its aim. Its aim is to connect the facts together in the most logical and plausible manner possible, and thus to aid the further advancement of our knowledge. Its "employment has its origin in the organization of the human mind, which handles abstract truths much less easily by themselves than by the help of an illustrative image. . . . . A hypothesis [or theory] can neither be proved nor disproved. It is merely a tool which is rejected when found to be no longer serviceable. . . . . What the 'real' nature of matter is, is to us a matter of complete ignorance, as it is of complete indifference." (OSTWALD-WALKER, *Outlines of General Chemistry* [London, 1895], pp. 5, 7.) A principle, on the other hand, does attempt to state the truth; it is a generalization and induction from a great number of known facts. When a fact is discovered which cannot be included under a principle, then that principle falls to the ground and ceases to be a principle. What was at first a theory may at length, by the accumulation of evidence, come to be a principle.

to whom we are indebted for the more important points. The student of this subject will find the following standard texts very helpful:

NERNST, W. *Theoretical Chemistry from the Standpoint of Avogadro's Rule and Thermodynamics.* Translated by C. S. Palmer. London, 1895.

OSTWALD, W. *Lehrbuch der allgemeinen Chemie,* comprising: *Verwandschaftslehre,* Leipzig, 1887; *Stoechiometrie,* Leipzig, 1891; *Chemische Energie,* Leipzig, 1893.

Solutions. Translated by M. M. P. Muir. London, 1891.

Outlines of General Chemistry. Translated by James Walker. London, 1895.

Manual of Physico-Chemical Measurements. Translated by James Walker. London, 1894.

The Principles of Inorganic Chemistry. Translated by Alexander Findlay. London, 1902.

REMSEN, I. *Principles of Theoretical Chemistry.* New York, 1897.

WALKER, J. *Introduction to Physical Chemistry.* London, 1899.

REYCHLER, A. *Outlines of Physical Chemistry.* Translated by J. McCrae. London, 1899.

DASTRE, M. A. "Osmose," in *Traité de physique biologique,* publié sous la direction de MM. D'Arsonval, Gariel, Chauveau et Marey. Tome I. Paris, 1901.

COHEN, ERNST. *Vorträge für Aerzte über physikalische Chemie.* Leipzig, 1901.

JONES, H. C. *The Elements of Physical Chemistry.* New York, 1902.

WHETHAM, W. C. D. *Solution and Electrolysis.* Cambridge, 1895.

KOHLRAUSCH, F., AND HOLBORN, L. *Das Leitvermögen der Elektrolyte.* Leipzig, 1898.

TRAUBE, J. *Physico-Chemical Methods.* Translated by W. L. Hardin. Philadelphia, 1898.

BLITZ, HENRY. *Practical Methods for Determining Molecular Weights.* Translated by Jones and King. Easton, Pa., 1899.

HAMBURGER, H. J. *Osmotischer Druck und Ionenlehre.* Wiesbaden, 1902.

CHAPTER I

MATTER AND ITS STATES

I. FUNDAMENTAL THEORIES OF THE NATURE OF MATTER

a) *The atomic theory.*—The whole structure of modern physical science is based upon the atomic theory. This theory supposes every mass of matter to be composed of numerous ultimate, indivisible particles, called atoms, which possess a peculiar attraction for one another. Atoms differ in the amount and nature of this attractive force, those of every chemical element being in this way different from those of every other; but all atoms of the same element, when under the same conditions, are exactly similar. Owing to their chemical attraction, atoms seldom exist free as such, but are prone to unite into groups, thus causing the neutralization of their mutual attraction. The groups so formed are called molecules. If the atoms composing the molecules of any substance are alike (*i. e.*, of the same element), the element is said to be in the molecular condition — as opposed to the atomic or nascent condition, in which the atoms are not united to one another, but exist free as such. When the atoms forming a molecule are of different chemical elements, a compound is said to be formed. The physical and chemical properties of molecules are very different from those of their component atoms, and they are also very different from those of any molecules which can be formed in any other way. But all molecules which are formed of the same elements and in the same manner are exactly similar under the same conditions. It thus appears that the smallest particle of a compound which can exist and still retain the properties of that compound is the molecule; break this up,

Austin College Library

and free atoms or new groups of atoms, with new properties, will result, the original compound having been destroyed by the process of separation. Atoms may also unite in such a way that their mutual attractive forces are but partially neutralized, thus forming incomplete parts of molecules, called ions. Under certain special conditions molecules may split into two or more ions, and some of these cases of ionization or dissociation, as the process of splitting is called, have proved very important in the development of the subject of osmotic pressure. In some cases an ion may consist of a single atom which has split off from some molecule. Briefly, then, acording to the atomic theory as now made use of, the nature of any mass is dependent upon that of its component particles, these particles being atoms, molecules, or ions. The same mass may contain, at the same time, all three kinds of particles.

b) *The kinetic theory of matter.*—According to the kinetic theory, the particles composing any mass, whatever their nature may be, are in constant motion. This necessitates their being considered, not as packed closely one against another to make up the mass, but as separated from one another by continuously varying spaces. The continuous motion of the particles is probably for the most part a vibratory motion. They are supposed to move in straight lines and in the same direction until a collision occurs, when they rebound according to the principle of the reflection of moving bodies. It thus becomes necessary to consider, for comparison, the average distance apart of these particles, or their average or mean free path. This has been demonstrated to be much greater than the diameter of a single particle.

A rough conception of the state of affairs within a mass of matter may be obtained by comparing the mass to a cage of angry bees. The insects in such a cage fly in straight

lines to and fro, striking against each other and against the walls of the cage, ever varying their distances apart, yet always remaining equally distributed throughout the cage, *i. e.*, always keeping their average distance apart the same.

Thus far nothing has been said of any restraining force to counteract in a measure the motion of the particles and keep them from flying apart indefinitely. Such a force might be roughly compared to the walls of the cage just referred to, for it is these restraining walls which prevent the indefinite enlargement of the swarm of angry insects. More accurately, the restraining force in the illustration is the sum of the reactions produced by the several impacts of the moving insects against the rigid walls. There is, indeed, such an active restraining force present in all masses of matter; it is ordinarily made evident, however, only in liquids and solids. This force is the cohesion of the particles themselves. It is probably akin to gravitation, in exhibited larger bodies, and is an inverse function of the square of the average distance apart of the moving particles. That is, the mutual attraction exerted by two particles decreases at the same rate as the square of their distance apart increases. It will thus be seen that this force becomes negligible at a comparatively small distance from any particle. But the particles of liquids and solids are so near to one another that their cohesive force is sufficient to overcome, to a certain extent, their energy of motion and to hold most of them within certain fixed limits of space.

The science of thermo-dynamics rests upon another supposition of the kinetic theory of matter, namely, that the temperature of any body is directly due to the kinetic energy of its vibrating particles. Since the mass of any particle remains constant, and the kinetic energy of any moving body is, at any instant, one-half the product of its mass and the square of its velocity ($KE = \frac{1}{2} M V^2$), it is seen that the average kinetic

energy of a particle varies with the square of its average velocity. We neglect here, as comparatively unimportant, all other forms of motion which a particle may possess, such as that of rotation, and consider only its translatory motion. Therefore, whenever the temperature of a quantity of matter is raised by any means, the average translatory velocity of its particles is increased. Now, the force of impact of a moving body is proportional to its momentum, which is equal to the product of its mass and velocity at the time of impact. But since one particle may strike another particle at any point in its free path, here again the average velocity must be considered. Therefore, since the mass of a particle is a constant quantity, any increase in the average velocity will cause a corresponding increase in momentum, and also in the force of impact. But the force of recoil is practically equal to the force of impact, and this latter force is the repellent force which tends to separate the particles. Thus, with rising temperature the repellent force is increased, the force of cohesion is more and more nearly overcome, and the particles become more and more widely separated. Also, with the rapid decrease in the cohesive force incident upon the increase in its acting distance, a limit is soon reached beyond which the force tending to cause separation is greater than the other, and the particles fly apart indefinitely. In this condition we say the substance is a gas. If it was a liquid or solid at the lower temperature, it has now been vaporized by heat.

II. THE THREE STATES OF MATTER

Matter exists in three states — the gaseous, the liquid, and the solid. In gases the kinetic energy of the particles is so great that the cohesive force is entirely overcome and the particles tend ever to increase their distance apart. From this it necessarily follows that a mass of gas in a closed

vessel will completely fill it, no matter if the vessel be many times the size of the original volume of gas. This is an observed fact.

If such a gas is gradually cooled (*i. e.*, allowed to do work on some other body and thus to part with some of the kinetic energy of its particles), a condition will be reached wherein the cohesive force is greater than the repellent, and the particles will remain together in a definite volume. As long as the two forces involved are nearly equal, the average free path will still be relatively great, and although the particles cling together, yet they will move very freely upon one another — a condition imperfectly simulated by the component grains in a mass of sand. In this condition the substance is said to be a liquid. Here the particles move so readily upon one another that a mass of liquid still takes the form of the containing vessel, as far as that is possible without increase in volume. In this regard liquids are very different from gases. Also, on account of the freedom of motion on the part of the particles making it up, and on account of the downward pull of gravity, the free surface of a liquid is usually approximately level. There are, indeed, certain phenomena of surface tension and adhesion which make it possible for free liquid surfaces to exist in other positions than the horizontal, but the present subject does not lead to a discussion of these. It is necessary to call attention, however, to the fact that, on account of the action of the cohesive force, a peculiar surface layer of particles is formed about a liquid mass, a sort of thin skin or film, which possesses considerable tensile strength, and which is much less easily penetrated than the internal mass.

By a continuation of the process of cooling (which must ever be thought of as a process of causing the body to give up kinetic energy by doing work, such as warming another cooler body) the liquid particles may be brought still closer

together, until cohesion becomes so strong, and hence the friction of particle upon particle so great, that the free movement upon each other just described comes practically to an end. The body is now a solid and will retain its form without surrounding walls. The particles are still in violent vibration, however. It should be stated here that the ideal gas, liquid, or solid does not exist; the hardest substances show some tendency to flow like liquids, and the most fluid substances exhibit some friction of their component particles upon one another.

CHAPTER II

DIFFUSION AND DIFFUSION TENSION

I. GASES

a) *Simple gases.*—As has been indicated already, it is a fundamental property of all gases that they tend to fill completely any vessel in which they may be inclosed. Thus, if a cubic centimeter of oxygen is measured out at ordinary temperature and at atmospheric pressure, and is then passed into a sealed vacuum chamber, it will completely fill the chamber, no matter how large the latter may be. This process of expansion is called diffusion. Of course, in diffusing, the particles of which the gas is composed become distributed throughout a greater space, and hence the gas becomes less dense. This property is often stated as follows: "The particles of gases tend to separate indefinitely."

Because of this tendency to expand, an outward pressure, called gas pressure, is exerted by a gas upon the walls of any chamber in which it may be confined. Gas pressure is supposed to be caused by the continuous bombardment of the walls of the inclosing vessel by the vibrating gas particles. If a gas be inclosed in a chamber with elastic walls, the size of the chamber will depend upon the number of particles of gas present (*i. e.*, its concentration) and upon the kinetic energy of the particles themselves (*i. e.*, its temperature). Thus, for any temperature and amount of gas, the distension of such a chamber will cease when the inward pressure, due to the resilience of the walls and to the pressure of the surrounding atmosphere (unless the chamber be in a vacuum), becomes equal to the outward pressure, due to the gas.

For a given amount of gas the pressure is constant at a constant temperature. But change in temperature means simply change in the kinetic energy of the particles. Therefore a rise in temperature must cause a corresponding increase in gas pressure, and a fall a corresponding decrease. Keeping the pressure constant, a rise in temperature produces an increase in volume, and *vice versa*. It has been found experimentally that the volume of a given mass of gas under constant external pressure varies with its absolute temperature (273° + the given temperature Centigrade). This is the principle of Gay-Lussac, sometimes called that of Charles.

But if, in the elastic chamber mentioned above, the temperature be kept constant and the resiliency of the walls be increased, thus increasing the external pressure on the gas, the volume will be decreased. As this occurs, however, the gas will increase in density, and continually more particles will strike unit area of the wall in unit time. Thus the internal pressure upon the bag will also be increased, until at length another state of equilibrium will be reached, wherein the external and internal pressures are again equal. But during the readjustment the volume of the gas has decreased. As long as the temperature (*i. e.*, the kinetic energy of the particles) is constant, an increase in external pressure produces a decrease in volume, and a decrease in external pressure an increase in volume. Experimentally it is demonstrated that the volume of a given mass of gas at a constant temperature varies inversely as the external pressure to which it is subjected. This is the principle of Boyle.

Still another principle has been discovered for gases. If the volume and temperature both remain constant, and if the number of particles is increased (*i. e.*, the concentration), the pressure will be correspondingly increased. It

is obvious from the theoretical consideration already presented that this must be true. In this case the kinetic energy of the particles is not altered, but their number has been increased, hence the increase in pressure. Also, for a given concentration and temperature, all gases exhibit the same pressure. This is called the principle of Avogadro. It is usually stated in a somewhat different way, namely: Equal volumes of gases, at equal temperature and pressure, contain the same number of particles. This principle holds rigorously true only for gases whose concentration is rather low. As a gas approaches the liquid state, the principle of Avogadro, and also those of Boyle and Gay-Lussac, have to be modified. They apply only to a theoretically perfect gas.

b) *Mixed gases.*— In a mixture of several gases each gas practically exerts its own pressure independently of the others. Thus the total pressure of a mixture of gases in a chamber is the sum of the pressures which would be exhibited were the gases separated and each put into a chamber of the same size as the first one, the temperature of course remaining constant. The pressures which would be thus shown are called partial pressures, and the above fact may be stated more directly, by use of this term, as follows: The total pressure of a gas mixture is practically the sum of the partial pressures of its component gases. As a gas nears the liquid state, this principle also breaks down in part, it too applying rigorously only to perfect gases.

Also, if two gases be brought together so as to form two horizontal strata in a chamber, diffusion of each gas will take place just as completely as if the other gas were not present. Particles of the lower gas will pass up from the lower stratum until that gas is equally distributed throughout the chamber. Downward diffusion of the upper gas will occur simultaneously, and the result of the two processes

will be a uniform mixture of the two gases. If this process of diffusion is obstructed by a wall placed between them, the pressures of both gases will of course be exhibited independently upon the opposite sides of this wall.

II. LIQUIDS

a) *Simple liquids.*—When a liquid is heated, the kinetic energy of its particles is increased, until at length the cohesive force which held them together is overcome; then they fly off from the main mass and tend ever to increase their distance apart. This is the process of vaporization by heat. As long as the temperature remains high enough, such matter will remain in the gaseous state. Also many substances which are usually liquids can be vaporized at ordinary temperatures. Water, alcohol, and ether are examples of this. This process, however, is a slow one. It is explained theoretically in this way: Although the majority of the liquid particles cannot break away from the main mass at ordinary temperatures, yet some of them, which reach the surface with greater kinetic energy than the others, do succeed in breaking through the firmer surface layer (see p. 7), and so escape as gas particles. If the chamber above the liquid be a closed one, so that the evaporated liquid cannot escape, evaporation soon apparently ceases. If some of the liquid particles come against the surface layer with sufficient force to pass through it, it is reasonable to suppose that, after escaping into the chamber above, some of them may again pass through this film in the opposite direction, and so re-enter the liquid. Here they come under the influence of the force of cohesion, which holds the liquid particles together, and, since they are unable to break forth at once, they remain in the liquid state. The number which thus re-enter the liquid will gradually increase as the pressure of the vapor (*i. e.*, the number of vapor particles, for the temperature is sup-

posedly constant) increases. Thus, an equilibrium will be established sooner or later, wherein the number of particles escaping from the liquid in unit time will be just equaled by the number re-entering it. That is, evaporation is just equaled by the opposite process, condensation. This is the condition when evaporation apparently ceases. The gas pressure with which the liquid particles escape is termed vapor tension. And when evaporation has apparently ceased, the gas pressure of the vapor in the space above the liquid is equal to the vapor tension which the particles exhibit in leaving the liquid surface. We have thus a means for measuring the vapor tension of any liquid.

If the temperature rises, the vapor tension rises correspondingly, following the principles of gases. If the external pressure upon the supernatant mass of vapor be increased, its gas pressure becomes greater than the vapor tension of the liquid, and condensation surpasses evaporation, thus decreasing the number of vapor particles — and hence the pressure due to them — until equality of tension and pressure is restored. If two such chambers in which the supernatant vapor is at different pressures be connected above the level of the liquid, the substance will distil over and condense in the chamber which has the lower pressure. This will continue until the two pressures have been equalized by the resulting change in the relative volumes occupied respectively by the two masses of liquid, and by the diffusion of the vapor particles themselves.

b) *Mixed liquids.*—If two different, equally miscible liquids are brought into contact with each other so as to form two horizontal strata, diffusion will take place in both directions, just as in the corresponding case with gases, but much more slowly on account of the friction and interference of the particles: and there will result a uniform mixture in which both kinds of particles are equally distributed

throughout. This tendency of one liquid to diffuse into another may be termed diffusion tension; it corresponds to the vapor tension exhibited by an evaporating gas. Diffusion in liquids is sometimes distinguished from that in gases by the use of the term "hydro-diffusion" to denote the former. They are, however, essentially the same thing. In the case just cited, each liquid develops a diffusion tension independently of the other. Of course, above such a mixture of liquids there will lie (if the chamber allow it) a stratum of gas mixture in which each of the two gases has its own vapor tension, just as though the other gas were not present.

III. SOLIDS

a) *Simple solids.*— Continuously raising the temperature of a solid may result in liquefying it and then in vaporizing the liquid thus formed; or vaporization may take place immediately, without the intervention of the liquid phase at all. In either case the particles break away from the solid mass and become more widely separated. With the process of liquefaction, however, we have no concern.

When vaporization of a solid takes place directly, it is called sublimation. Gum camphor, naphthalene, and ice below the temperature of melting exhibit this phenomenon. If the vapor particles are prevented from escaping, an equilibrium between vapor and solid is ultimately reached, at which sublimation apparently ceases. At such a point sublimation is just equaled by condensation. The whole process is analogous to that of evaporation from free liquid surfaces. The pressure of the vapor surrounding a solid mass of the same substance, when equilibrium is reached, may be termed, as in liquids, vapor tension.

b) *Diffusion of two solids.*—If two solid masses of different substances are brought together with their adjacent faces in close contact, there can be demonstrated, in some

cases at least,[1] a diffusion of the substances into each other. The process goes on with extreme slowness, however, and the details need not be stated here.

[1] W. SPRING, "Ueber die chemische Einwirkung der Körper in festem Zustande," *Zeitschr. f. physik. Chem.*, Vol. II (1888), pp. 536-8; IDEM, "Ueber das Vorkommen gewisser für den Flüssigkeits- oder Gaszustand charakteristischen Eigenschaften bei festen Metallen," *ibid.*, Vol. XV (1894), pp. 65-78; W. ROBERTS-AUSTEN, "On the Diffusion of Gold in Solid Lead at the Ordinary Temperature," *Proceed. Roy. Soc. London*, Vol. LXVII (1901), pp. 101-5.

CHAPTER III

LIQUID SOLUTIONS

"SOLUTIONS are homogeneous mixtures — mixtures which allow no separation of their components by mechanical means. The ability of gases to form such mixtures is unlimited, that of liquids is limited."[1] Solid solutions also exist, but have not yet been shown to play any part in physiology; therefore they need not be considered here. Gas mixtures have already been discussed. There remains, then, only the subject of liquid solutions — a very important subject in the study of physiology.

I. SOLUTIONS OF LIQUIDS IN LIQUIDS

Not nearly all liquids are readily miscible to form solutions. Many are nearly — perhaps quite — insoluble in one another. Again, many liquids are mutually soluble in all proportions (*e. g.*, water and alcohol); others are so only within certain limits.

When a mixture of two liquids is considered as a solution, the liquid which preponderates is called the solvent and the other the solute. If such a solution were brought into contact with a mass of the pure solvent, diffusion of the solute would take place into the pure solvent until the solute were uniformly distributed throughout both layers. At the same time the pure solvent would diffuse into the solution. Of course, the interchange of particles between two such layers would not cease when uniformity of constitution had been attained throughout; it would still go on, but would cease to be apparent, having become simply the continuous motion of the particles composing the uniform mixture. At

[1] OSTWALD-WALKER, *Outlines of General Chemistry* (London, 1895), p. 117.

the beginning of such a process of mixing, however, a definite diffusion tension exists and can be demonstrated — a diffusion tension produced on the one hand by the solute, and on the other by the solvent. These diffusion tensions are identical in their nature with those spoken of in the last chapter. They increase in amount with rise in temperature, and, in case there are several solutes, each one has its own diffusion tension. These facts are found to be fundamental in the consideration of osmotic pressure.

Above any liquid mixture contained in a closed jar which it does not fill, there will be a gas mixture of the vapors of the solvent and of the several solutes. Each body will have its own vapor pressure, and the total pressure of the gas mixture will be the sum of its partial pressures.

II. SOLUTIONS OF GASES IN LIQUIDS

Gas solutes behave in the same manner as that just described for liquid solutes. The amount of gas going into solution, when a mass of it is brought into contact with a mass of liquid solvent, increases with the temperature and pressure. Diffusion pressures of solvent and solute are developed here also, and are constant for a given temperature; they also vary with the absolute temperature. There may be several gaseous solutes in the same solution, and in this case each develops its own diffusion tension in the solvent. Above such a solution there will be a gas mixture of the vapor of the solvent and of the several solutes. Interchange of particles will go on continually between the gas solution above and the liquid solution below, but will not be apparent for reasons similar to those expressed above for liquid solutes. Also, if a solution containing a gas solute be brought into contact with a mass of the pure solvent, diffusion will take place of both solvent and solute, each developing its own diffusion tension in its own direction, just as

in the corresponding case with a liquid solute. Equilibrium and an apparent stoppage of diffusion will be brought about when diffusion is equal in both directions.

III. SOLUTIONS OF SOLIDS IN LIQUIDS

If a crystal of sugar or salt be put into the water, it dissolves. This process of dissolving consists in the flying off of particles into the water, just as the process of vaporization of a mass of naphthalene consists in the flying off of particles into the air. After the particles of the dissolved substance (solute) are once free from the solid mass, they behave in an entirely different manner from that which characterized them before. While they were in the crystal they clung together by cohesion. Now they tend to separate as much as possible within the limits of the solvent.[1] They may or may not pass the surface of the solvent and enter the air as a gas, but within the solvent they continue to diffuse until they are uniformly distributed. Diffusion of the solute in its solvent takes place much more slowly than does gas diffusion, but in the end it is just as complete. Thus it is evident that within the volume occu-

[1] T. GRAHAM, "Ueber die Diffusion von Flüssigkeiten," *Liebigs Ann.*, Vol. LXXVII (1851), pp. 56–89 and 129–60; see also *ibid.*, Vol. LXXX (1851), pp. 197–201. The following references may serve to put the reader into contact with the literature of diffusion: T. GRAHAM, "Supplementary Observations on the Diffusion of Liquids," *Phil. Trans. Roy. Soc. London*, 1850, pp. 805–36; A. FICK, "Ueber Diffusion," *Pogg. Ann.*, Vol. XCIV (1855), pp. 59–86; T. GRAHAM, "Anwendung der Diffusion der Flüssigkeiten zur Analyse," *Liebigs Ann.*, Vol. CXXI (1862), pp. 1–77; F. VOIGTLÄNDER, "Ueber die Diffusion in Agargallerte," *Zeitschr. f. physik. Chem.*, Vol. III (1884), pp. 316–35; J. D. R. SCHEFFER, "Untersuchungen über die Diffusion einiger organischen und anorganischen Verbindungen," I, *Ber. d. deutsch. chem. Gesellsch.*, Vol. XV (1882), pp. 788–801; II, *ibid.*, Vol. XVI (1883), pp. 1903–17; W. NERNST, "Zur Kinetik der in Lösung befindlichen Körper," *Zeitschr. f. physik. Chem.*, Vol. II (1888), pp. 613–37; J. D. R. SCHEFFER, "Untersuchungen über die Diffusion wässeriger Lösungen," *ibid.*, pp. 390–404; S. ARRHENIUS, "Untersuchungen über Diffusion von in Wasser gelösten Stoffen," *ibid.*, Vol. X (1892), pp. 51–95; L. LIEBERMANN UND S. BURGARSZKY, "Beiträge zur Theorie der wässerigen Lösungen von Salzgemischen," *ibid.*, Vol. XII (1893), pp. 188–95; A. NACCARI, "Sulla pressione osmotica," *Atti della Reale Accad. dei Lincei*, Ser. 5, Rendiconti, Classe di Scienze fisiche, matemat. e naturali, Vol. II (1893), 1 Semestre, pp. 238–9, and 2 Semestre, pp. 136–8; R. HÖBER, "Ueber Concentrationsänderungen bei der Diffusion zweier gelöster Stoffe gegen einander," *Pflügers Archiv*, Vol. LXXIV (1899), pp. 225–45.

pied by the solution, dissolved particles exhibit at least one of the fundamental properties of gas particles, namely, that of indefinite diffusion. It will be gathered from what was said under the preceding headings that the same is true for liquid and gas solutes.

As in gases and in solutions with liquid and gas solutes, this tendency of the solute to diffuse (diffusion tension) may be measured. It is found that, for the same temperature and volume, the same number of particles of different solutes gives always the same diffusion tension. Thus the solute in such a solution exhibits another principle of gases, namely, that of Avogadro. This, too, is true for liquid and gas solutes. The principle does not hold rigorously for very concentrated solutions. There is developed here also a diffusion tension on the part of the solvent, which varies with temperature just as does that of the solute.

If two solutions containing different concentrations of the same solute in the same solvent are brought into direct contact, it is found that diffusion of solvent and solute will at length equalize the concentrations of the two solutions, so that the solute particles will at last be equally distributed throughout the combined volume. Therefore diffusion of solute particles must be more rapid from the stronger to the weaker of the two solutions than in the opposite direction. That is, the diffusion tension of the solute is greater from a higher concentration to a lower than from a lower to a higher. But the diffusion tension of the solvent is greater in the direction from the lower concentration to the higher. This is also true in the case of gas and liquid solutes. When reference is made to the "concentration" of a solution, the concentration of the solute is always meant.

9830

IV. TERMINOLOGY FOR SOLUTIONS OF DIFFERING CONCENTRATION

To designate different concentrations of solutions, the most common method among physiological writers has been, until quite recently, that of percentage. An example of this method will explain its use. A solution is said to be a 5 per cent. solution of a certain solute in a certain solvent when it is composed of five parts by weight of solute to ninety-five parts by weight of solvent. But solutions of different solutes in the same solvent depend for their physical properties upon the relative number of solute particles which they contain per unit volume. A glance at a table of atomic weights will make it clear that any method by weights which has as its basis the percentage system cannot readily be adapted to a discussion of the relative number of molecules contained in equal volumes of solutions of different solutes. Atomic weights, and therefore molecular weights, cannot readily be compared in terms of percentage. As long as physiologists persist in using this antiquated method in the preparation of their solutions, so long will they fail to arrive at any far-reaching principles concerning the chemical and physical nature of the substances used.

A more scientific method is that based on the relative number of particles of solute in unit volume of solution. We cannot, of course, actually count the molecules of any substance, but from a knowledge of the relative weights of the molecules of different bodies it is easily possible to get several masses of different substances, each of which will contain approximately the same number of molecules. The weights of such masses must be to each other as the molecular weights of the respective substances. For instance, 342 grams of cane sugar (mol. wt. 342) must contain the same number of molecules as 180 grams of glucose (mol. wt. 180), for the molecular weights give the relative weights of the

two different molecules. Now, if these two masses are placed in equal volumes of solution, both solutes ought to show the same diffusion tension. This, indeed, is found to be true, and the same principle has been shown to be true, as far as experiment has gone, for solutions of all substances which do not conduct electricity (non-electrolytes). Solutions of non-electrolytes which contain the same number of molecules per unit volume have the same diffusion tension (at the same temperature) and are physically similar. Solutions which conduct electricity exhibit this principle only in a general way. Their departures from it and the reasons therefor will be discussed in the next chapter.

The number of grams of a substance represented by its molecular weight is called a gram-molecule. Gram-molecules of all substances contain, then, the same number of molecules. If a gram-molecule of some substance be put into solution, and then this be diluted to one liter, there results a solution which can reasonably be used as a standard. Such a solution is often termed a molecular solution. Thus, a molecular solution of potassium nitrate (mol. wt. 101) is 101 grams of the salt in a liter of solution. It is as though the substance had been vaporized and the resulting gas occupied a volume of one liter.

But the analytical chemist has found it convenient to use another solution as a standard. He dissolves, to form a liter of solution, as many grams of the substance in question as will react chemically with a gram-molecule of a monovalent compound. This amount of substance is termed a gram-equivalent. A gram-equivalent of sulphuric acid (H_2SO_4) will just neutralize a gram-equivalent of potassium hydroxide (KOH) or will just decompose a gram-equivalent of sodium chlorid (NaCl); but it takes two gram-equivalents of either of the last-mentioned compounds to react completely with a gram-molecule of sulphuric acid. It follows that "gram-

equivalent" and "gram-molecule" are synonymous terms in the case of monovalent compounds, and that a gram-equivalent of a bivalent compound is one half of its gram-molecule, of a trivalent compound one-third, etc.

A solution made up so as to contain in one liter a single gram-equivalent of solute is termed an equivalent normal, or simply a normal, solution. Unfortunately there is a usage which terms a molecular solution normal, thus giving rise to ambiguity for all but monovalent solutes. This ambiguity can be avoided only by the careful definition of the term "normal" by each author using it.[1] For all neutral organic compounds, such as the sugars, and also for monovalent electrolytes, a gram-equivalent is the same as a gram-molecule, and a normal solution must be a gram-molecule in a liter volume. Thus the sugar solutions described in a previous paragraph are both normal solutions. No ambiguity can arise from the use of the term in reference to such compounds.

Regarding acid salts (such as $KHSO_4$, for example), there is a difference of opinion as to what should be denoted by gram-equivalent. Some hold (*e. g.*, Kahlenberg) that in the salt just mentioned gram-equivalent and gram-molecule are identical. Thus, such a salt might be regarded as a monobasic acid. On the other hand, Sutton, Fresenius, Dandeno, and others regard a gram-equivalent of $KHSO_4$ as one-half a gram-molecule. Thus, an equivalent solution of this salt would contain only one-half gram of hydrogen; the salt is to be regarded as a monobasic acid, one-half of whose hydrogen has been replaced. It seems that the latter is the more truly scientific position.

[1] For an account of confusion (partly imagined) which has arisen from a lack of attention to such definition of these terms, see J. B. DANDENO, "The Application of Normal Solutions to Biological Problems," *Bot. Gaz.*, Vol. XXXII (1901), pp. 229–37. Also see "Open Letters," one from LOUIS KAHLENBERG, and an answer from DANDENO, *ibid.*, p. 437.

CHAPTER IV

IONIZATION

I. IONIZATION OF GASES

From the hypothesis of Avogadro it would be expected that, if a gram-molecule of ammonia and a gram-molecule of ammonium-chlorid vapor were put into chambers of the same size, the pressures exhibited at the same temperature by the two gases would be equal. The latter substance, however, shows a far greater pressure. Now, since the kinetic theory supposes that gas pressure is due to the kinetic energy of its particles, and that the kinetic energy of any particle is dependent only upon the temperature to which it is subjected, we must either reject the theory when we come upon such a case as that just cited, or we must conclude that there are, in the mass of ammonium chlorid, a greater number of particles than in that of ammonia. Several lines of experiment and of reasoning seem to point to this as the true condition of affairs. The number of molecules is the same in both masses of gas, but in the ammonium chlorid it is supposed that many of the molecules split apart into ammonia and hydrochloric-acid ions (NH_3 and HCl), and that, for producing pressure, the ions are as active as would be the same number of molecules. In this way, if all the molecules were dissociated, the pressure should be twice that required by the theory. The ammonium molecule seems not to dissociate at ordinary temperatures. Many gases exhibit the phenomenon just described; usually ionization is not nearly complete and the pressure is simply raised above its theoretical value. As the gas becomes more concentrated, dissociation becomes less and less complete.

The theory just given is called the theory of dissociation. There are other theories to account for these phenomena, but this has the widest acceptance at the present time and serves the purpose of the physiologist better than any other yet advanced.

II. IONIZATION OF SOLUTES IN LIQUID SOLUTIONS

It has been found that a phenomenon similar to the one just described occurs in dilute aqueous solutions of electrolytes. These solutions uniformly give a higher diffusion tension than the one required by the theory. The explanation is the same as that given above;[1] if ammonium chlorid, for instance, be put into aqueous solution, it has been shown that some of the molecules ionize, thus increasing the diffusion tension of the solute. The more dilute the solution, the greater is the proportion of molecules dissociated, and at infinite dilution a limit would be reached at which complete dissociation would occur. This theory is named for its originator, Arrhenius. In very weak solutions it is found that practically all the molecules are ionized. If several electrolytes are contained in the same solution, ionization occurs in all of them, but not always to the same degree as if there were but one solute; the presence of other molecules and ions seems to influence the amount of dissociation. This subject has not yet been sufficiently investigated to permit the formulation of a general principle. Where a solution contains several different kinds of ions, it is found that the velocity of diffusion of some ions is much greater than that of others.

Only those substances which conduct electricity when in solution are dissociated. The whole theory of primary batteries and of electric conduction by liquids depends upon this principle. Enough has been said, however, to prepare the way for what is to follow.

[1] S. ARRHENIUS, "Ueber die Dissociation der in Wasser gelösten Stoffe," *Zeitschr. f. physik. Chem.*, Vol. I (1887), pp. 631–48.

9800

CHAPTER V

OSMOTIC PHENOMENA

I. OSMOTIC PRESSURE OF THE SOLUTE

a) *Non-electrolytes.*—If a parchment-paper bag be filled with aqueous sugar solution and, after the opening has been sealed, the bag be submerged in water, the walls will soon be distended by an internal pressure. If the original solution is strong enough, the walls will be stretched to their limit of extensibility, and at last ruptured. If, during the distension of the bag, the water around it be tested, it will be found to be nearly or quite free from sugar; after the bag is ruptured, however, we find the sugar diffusing rapidly to the limits of the water. Therefore parchment paper hinders greatly the diffusion of the sugar, *i. e.*, it is only slightly permeable to dissolved sugar molecules. This fact forms the basis for an explanation of the phenomenon of distension and rupture just mentioned. In tending to diffuse indefinitely, the dissolved molecules (there is no dissociation in the case of sugar and other non-electrolytes) bombard the walls of any chamber in which they may be inclosed.[1] The fact that they possess this property of indefinite diffusion only when within the limits of the solvent makes it necessary that such a chamber be surrounded by the pure solvent, and that the solvent permeate its walls. The pressure thus produced upon the walls of the bag is really the diffusion tension of the solute. If diffusion could take place without obstruction, this pressure would not be made apparent, but would exist none the less. The water itself exerts but little pressure upon the walls of the bag, since

[1] J. H. VAN'T HOFF, "Die Rolle des osmotischen Druckes in der Analogie zwischen Lösungen und Gasen," *Zeitschr. f. physik. Chem.*, Vol. I (1887), pp. 481–508.

these are almost freely permeable to it, but a diffusion tension of water exists and can be demonstrated in other ways. The internal pressure of dissolved sugar molecules forces the walls of the bag outward through the water, just as a cloth bag may be distended under water by the expansion of wire springs inside of it. Of course, in such a process of distension water enters the bag from without, the bag being permeable to that substance. If in either case the bag is not strong enough to bear the pressure, it will burst when its limit of extensibility is reached. If the parchment bag is strong enough to withstand the pressure developed within, an equilibrium will be established, just as in the case of the expanding gas described in chap. ii. In this condition of equilibrium the inward pressure (due to the resilience of the walls of the bag) is just equaled by the outward pressure (due to the bombardment of the walls by the solute particles).

Such a membrane as parchment paper, which allows the solvent to pass, but greatly retards or prevents the passage of solute particles, is said to be semi-permeable; and the pressure which such a membrane makes evident under the conditions just described is the osmotic pressure of the solute. This is merely the diffusion tension of the solute, made evident by the opposition of the membrane. All possible gradations exist between membranes which are freely permeable to solutes and those which retard them or are impermeable to them. It needs to be noted here, however, that a theoretically perfect semi-permeable membrane has not been found. The best ones which have been tested allow some passage of solute particles. Many animal membranes are nearly semi-permeable in certain solutions, pig's bladder being often used. A membrane of copper ferrocyanid is almost perfectly semi-permeable in aqueous sugar solution, but it is permeable to certain salts, *e. g.*, potassium

nitrate. The term "semi-permeable," therefore, must be used with reference to a particular solute and its solvent.

Such membranes as that of copper ferrocyanid are termed "precipitation membranes;" they are formed by precipitation from two solutions which react chemically. If a solution of potassium ferrocyanid and one of copper sulphate be brought together within the walls of a porous clay cup, such a membrane (composed of copper ferrocyanid, $Cu_2Fe(CN)_6$) will be precipitated within the clay walls. The membrane is then supported by the clay, and the whole cup may be used for osmotic determinations. Another precipitation membrane is that formed by gelatin and tannic acid.

b) *Electrolytes.*—Osmotic pressure is found to be abnormally high in solutions of electrolytes. This is one of the facts from which the conclusion was drawn that in these solutions the diffusion tension of the solute is abnormally great, and hence that dissociation occurs. When the amount of ionization which takes place in any solution is taken into account, it is found that these solutions are only apparent exceptions to the general rule of osmotic pressure. In this case we can no longer say that the pressure is due to the bombardment of the membrane by the solute molecules, but by the solute *particles*, meaning thereby both molecules and ions. Solutions having the same number of solute particles per unit volume have, at the same temperature, the same osmotic pressure. As far as it has been carefully tested, this principle has been found to hold for all somewhat dilute solutions.

c) *Colloids.*—According to their behavior when in solution, substances have been classified as crystalloids and colloids. Crystalloids produce an osmotic pressure which is practically equal quantitatively to the gas pressure which would be produced by the same number of gas particles as there are of solute particles, occupying the same volume as

the solution and possessing the same temperature. There is, however, a group of substances soluble in water, which do not produce osmotic pressure at all, or produce it in a very slight degree. These are the so-called colloids, such as gelatin, gum, silicic acid, aluminium hydroxid, etc. These substances have very large molecules which diffuse with exceeding slowness and seem to encounter great resistance in passing through water. Colloids in their relation to crystalloids bid fair to become very important in the advance of physiological knowledge.

d) *Osmotic pressure in general.*— Most solutes which produce osmotic pressure when in solution are either solids or liquids at ordinary temperatures, when not in solution. But a gas in solution may also produce osmotic pressure if a suitable membrane is employed.

Osmotic pressure being, in its origin, perfectly comparable to gas pressure, the various principles established for gas pressure have been found to hold for osmotic pressure. The principles of Boyle, of Gay-Lussac, and of Avogadro, developed for gases, have all been extended so as to include substances in solution. Here is convincing evidence that a solute, as long as it is in solution, is essentially a gas occupying the volume of the solution. The solvent merely provides conditions under which the pseudo-vaporization which we call solution can take place. Osmotic pressure is independent of the solvent and is dependent only upon the number of particles of solute (*i. e.*, its concentration) and upon their kinetic energy (*i. e.*, their temperature). The nature of the solute is immaterial, the number of particles (molecules or ions) per unit volume being, as far as is known, the only essential factor.

Where several substances that do not react chemically are in very dilute solution, the osmotic pressure of the mixture is the sum of the pressures which would be exhibited were

each of the different solutes dissolved separately to form a volume of solution equal to the original volume. As in gases, these latter pressures are termed partial pressures. This principle may be formulated again as follows: The total osmotic pressure of a dilute solution of mixed solutes is the sum of the partial osmotic pressures of the component solutes. As was seen in the last chapter, however, for more concentrated solutions this principle does not seem to hold. But more work needs to be done here before we may be positive.

The principles of Boyle and Gay-Lussac would hold perfectly true only for an ideal gas, *i. e.*, one without any friction between its particles. Such a gas does not exist, although hydrogen approaches this condition very nearly. These principles, however, hold very nearly true for all ordinary gases as long as they are not nearing the point of condensation into a liquid. But, as has been stated, in the vicinity of this critical point, whether it be approached because of increase in pressure or fall in temperatnre, they do not hold true. In the study of osmotic pressure it is found that a similar breaking down of the same principles occurs when the solute becomes too concentrated. At high concentration the principles of gas pressure no more apply to osmotic pressure than they do to gas pressure itself.

Just what is the action of the membrane in osmotic phenomena is not known. In many respects it acts like a sieve or filter, to prevent the passage of large particles, but allow smaller ones to go through unhindered. In some cases, however, the chemical nature of the membrane seems to come into play; it seems to react chemically with the solute particles, taking them up on one side and giving them off on the other. But for a discussion of the principles and general phenomena of osmotic pressure, a knowledge of the exact method by which the membrane acts seems not to be

essential. A brief discussion of the different theories which have been proposed to account for the action of the membrane will be presented in connection with the treatment of the protoplasmic membranes of vegetable cells (see p. 80).

II. DIFFUSION TENSION OF THE SOLVENT

The diffusion tension of the solvent has been mentioned several times during the discussion of solutions, but it is thought well to bring together in this place the ideas concerning it.

If the vapor tension of pure water be determined and then that of an aqueous salt or sugar solution, it will be found that the latter is invariably less than the former, and this in proportion to the concentration of the solution. Therefore it must be that particles of the solute hinder the escape of the solvent molecules. The moving particles of solute perhaps bring this about merely by moving into the path of solvent molecules which would otherwise leave the liquid. It is probable that there exists also an attraction between the particles of solute and solvent.

If two solutions whose concentrations are different be brought into direct contact, as by placing a weak sugar solution over a stronger one, phenomena similar to those just discussed may be detected. Water molecules pass through the common surface in both directions. They are not vaporized, for they remain in the liquid state, but they diffuse as liquid molecules. Under such conditions the diffusion of the solvent is always found to be greater from the weaker to the stronger solution than in the opposite direction; it will be remembered that the most rapid diffusion of the solute takes place from stronger to weaker solution. There is, therefore, a difference in the energy of diffusion (diffusion tension) of the solvent in the two solutions. This corresponds to their difference in vapor tension just de-

scribed. The diffusion tension of the solvent is greatest in the pure solvent and decreases as the concentration of the solution increases.

If there were available a membrane permeable to the solute, but impermeable to the solvent, this diffusion tension of the solvent might be directly measured. It would be an osmotic pressure similar to that occasioned by the solute molecules, but of much greater magnitude and in the opposite direction. Though there is no membrane which will make this pressure evident, most of the phenomena of osmotic pressure show that it exists. No membrane can allow the solvent particles to pass absolutely without friction. Thus the question arises: Why is not this pressure of the solvent made evident to a degree equal to the amount of force needed to overcome this friction? The answer is, obviously, that the pressure produced by the solvent on one side of the membrane is practically equaled by that on the other side. In solutions where the principles of osmosis hold true, the dilution of the solvent, due to the presence of the solute, is negligible.

The following explanation of osmotic pressure has been given by various authors. The quotation is from Davenport:[1] "Upon the side containing the greater number of molecules of salt [solute] fewer water [solvent] molecules will in a given time strike the membrane than upon the other side; and since the number passing through is proportional to the number striking, relatively fewer molecules of water will consequently pass out, and so there will be a resultant flow of water to that side; and if the mass of water is confined, it will exert great pressure." This explanation is untenable for several reasons. Not nearly all solutions occupy more space than the original mass of pure solvent from which they were prepared. If to exactly a liter of water be added a

[1] C. B. DAVENPORT, *Experimental Morphology*, Vol. I (New York, 1897), p. 71.

given quantity of some solute, it cannot be told *a priori* whether the resulting solution will occupy the same volume as the original solvent, or a greater or less volume. Into this matter it is unnecessary to go farther than to add that osmotic pressure may be demonstrated as readily in solutions occupying less volume than the original solvent as in those occupying more. It is obvious that in the former case there must be a greater number of solvent particles per unit volume than in the pure solvent. Hence, if the above explanation can be retained, there should be no osmotic pressure developed in such a solution; indeed, it should appear on the side of the pure solvent.

But even if it were possible that the entrance of solvent particles into the solution was due to such a difference in concentration of the solvent on opposite sides of the membrane, the explanation just quoted would fail. It is inconceivable that the osmotic membrane should be more permeable to solvent particles moving in one direction than to those moving in the other, and it thus becomes impossible to suppose that solvent particles which can pass the membrane in one direction "will exert great pressure" upon it in the other. Great hydrostatic pressure cannot be maintained in a sieve, nor can osmotic pressure be maintained upon a membrane by a solvent to which it is permeable. Any difference between the solvent pressures on the two sides of an osmotic membrane must be very rapidly destroyed by diffusion of the solvent through the membrane.

III. EXPERIMENTAL DEMONSTRATION OF OSMOTIC PRESSURE

If a parchment-paper bag, like the one used in the illustration of osmotic pressure, were fixed in a firm cage, so that it could not expand except on one side, and were then filled with solution and submerged in pure solvent, bulging would occur on the free side. It would make no difference whether

this free side were serving as an osmotic membrane or not, for pressure produced anywhere in the bag must be transmitted equally and undiminished to all parts of the surface, in accordance with Pascal's principle of transmission of pressure in fluids. This transmission would be accomplished by the fluid as a whole, solvent and solute acting together. If, however, the portion where the bulging is supposed to take place be permeable to the solvent, the immediate pressure which affects the membrane must be due to the solute. Thus it comes to the same end if we consider the pressure as transmitted by the solute, acting like a gas; for increased energy of solvent particles will be transmitted to the solute particles with which they come in contact.

A very common mode of demonstrating the existence of osmotic pressure is the following: A piece of animal bladder or parchment paper is tied tightly over the expanded end of a thistle tube, and the bulb is filled with molasses, or a strong aqueous sugar or salt solution. Then the tube is fastened upright, the bulb being immersed in water so that the liquids within and without have a common level. After a time it is observed that the solution has risen in the tube, often to the height of a meter or more if the tube is sufficiently long. The diffusion tension of the solute particles within the bulb is of course operative in every direction, but osmotic pressure is developed and made apparent only within the membrane. This pressure is transmitted through the liquid to all parts of the surface of the solution. But the only part of this surface which is free to move, after the limit of extensibility of the membrane is reached, is the free surface in the stem of the tube. We have seen that the free surface of a liquid is bounded by a peculiar layer or film. Upon this film the transmitted osmotic pressure is effective, just as though the film were a piston closely fitting within the bore of the tube. In this case, since the surface layer is nearly impermeable to

Austin College Library

liquid solvent, the pressure of solvent particles may be immediately effective. The surface film is lifted by the pressure exerted from below, as a piston in such a position might be lifted by wire springs situated within the bulb; and, in rising, be the change in level ever so slight, it increases the volume of the solution in the tube, thus decreasing the diffusion tension of the solvent within this solution, and also overcoming to some degree the atmospheric pressure on the free surface of the solution; and water enters through the permeable membrane below. The entrance of the water is due mainly to the diffusion tension of the solvent and in part to hydrostatic pressure. In the latter sense the pushing up of the surface film acts like the raising of a piston in a pump. With a closed water manometer, or an open one of mercury, a pressure far surpassing that of an atmosphere may be obtained where the membrane used is sufficiently strong. Of course, in such cases hydrostatic pressure as a cause for the ascent of the column is to be ruled entirely out of consideration.

Other methods of demonstrating osmotic pressure are in use, but the explanation just given may be applied, *mutatis mutandis*, to any of them.

CHAPTER VI

MEASUREMENT AND CALCULATION OF OSMOTIC PRESSURE

I. MEASUREMENT OF OSMOTIC PRESSURE

a) *Direct method.*—The direct method of measurement of osmotic pressure is very difficult of operation, and determinations thus made are exceedingly tedious processes. Nor is this method susceptible of sufficient accuracy to recommend it to physiologists. But since it is the classical method used by Pfeffer[1] in his original investigation of the subject, and since it has been used since that time by physical chemists in establishing the principles by which indirect methods become available, it will be described here at some length.

A membrane of copper ferrocyanid is precipitated within the walls of a cup or bulb of porous clay (a filter bulb serves admirably) by filling the bulb with a solution of potassium ferrocyanid and surrounding it with one of copper sulphate. The bulb should first be thoroughly cleaned and freed from air by boiling for some time in water. When the membrane is well formed (which occurs after fifteen to forty hours), the cup is filled with the solution to be tested, closed with a rubber stopper bearing a mercury manometer, and immersed in water. The osmotic pressure rises for a number of hours, being indicated by the rise in the mercury column, and at last, when the membrane has been ruptured somewhere, begins to descend again. The maximum reading of the mercury column is taken as the osmotic pressure of the solution. The difficulty of the method lies in getting

[1] W. Pfeffer, *Osmotische Untersuchungen*, Leipzig, 1877.

a perfect semi-permeable membrane which will withstand the high pressures developed. A number of determinations for the same solution are necessary in order to eliminate erratic cases.

The direct method of measurement has just been brought again into prominence by the work of Morse and Horn.[1] These authors have succeeded in forming much more perfect membranes in porous clay cups than have ever been produced before. Air is first swept out of the pores of the cup by an "endosmotic" current. The cup is filled with a weak solution of K_2SO_4 and immersed in a vessel of the same solution until the outer level is near the margin of the cup. Then a current from a dynamo is passed between a cylindrical copper electrode surrounding the cup and a platinum electrode within it. As the liquid rises in the cup, it is removed, and in a short time the air is all removed from the porous clay. Then the cup is filled with $K_4Fe(CN)_6$ and immersed as before, but now in a solution of $CuSO_4$. The current is passed again, and thus the $Fe(CN)_6$ ions are driven into the clay from one side, while the Cu ions are forced in from the other. The resistance of the cup gradually rises as the membrane is formed, being from fifteen hundred to three thousand ohms at the time when the membrane is considered as complete.[2]

b) *Indirect methods.*—Owing to the difficulties encountered in the use of the direct method just described, an indirect method is usually resorted to. These indirect methods depend upon the general principles, that depression

[1] H. N. MORSE AND D. W. HORN, "The Preparation of Osmotic Membranes by Electrolysis," *Am. Chem. Jour.*, Vol. XXVI (1901), pp. 80–86.

[2] The most recent investigations into the nature of precipitation membranes are as follows: G. TAMMANN, "Ueber die Permeabilität der Niederschlags-Membranen," *Zeitschr. f. physik. Chem.*, Vol. X (1892), pp. 255–64; P. WALDEN, "Ueber Diffusionserscheinungen an Niederschlags-Membranen," *ibid.* (1892), pp. 699–732; J. H. MEERBURG, "Zur Abhandlung Tammanns: Ueber die Permeabilität der Niederschlags-Membranen," *ibid.*, Vol. XI (1893), pp. 446–8.

of the freezing-point, elevation of the boiling-point, decrease of the vapor tension of solutions, and osmotic pressure are all related phenomena, and may be obtained one from the other for any given solution.[1] Three of the most satisfactory methods for determining osmotic pressure in this way will be briefly described here:

1. The freezing-point method: The freezing-point of a solution is always lower than that of the pure solvent. This depression of the freezing-point is proportional to the number of solute particles present, and therefore to the osmotic pressure.

The depression of the freezing-point can best be determined by means of Beckmann's apparatus,[2] which may be found described in any of the texts on physical chemistry. A determination of the freezing-point is first made for distilled water; this is followed by a determination for the solution to be tested, care being taken not to disturb the adjustment of the thermometer between the determinations. The difference between the two observations will be the required depression, which may be denoted by Δ_f. The relation between this quantity and the osmotic pressure is expressed, for aqueous solutions, by the following equation:

$$P_f = 9173.2\,\Delta_f\ ,^{3}$$

wherein P_f is the osmotic pressure at the freezing-point of the solution, measured in millimeters of mercury.

The osmotic pressure at any desired temperature other than the freezing-point, say T in the absolute scale, may be obtained by applying the principle of Gay-Lussac, which

[1] J. H. VAN'T HOFF, "Die Rolle des osmotischen Druckes in der Analogie zwischen Lösungen und Gasen," *Zeitschr. f. physik. Chem.*, Vol. I (1887), pp. 481–508.

[2] E. BECKMANN, "Ueber die Methode der Molekulargewichtsbestimmung durch Gefrierpunktserniedrigung," *ibid.*, Vol. II (1888), pp. 638–45.

[3] NERNST-PALMER, *Theoretical Chemistry* (London, 1895), p. 132. The pressure here is reduced from atmospheres to millimeters of mercury.

holds for osmotic pressures of dilute solutions. This operation is expressed in the following:

$$P_T T_f = P_f T \ ,$$

in which P_T is the osmotic pressure, in millimeters of mercury, at required temperature T (absolute), and T_f is the absolute freezing-point of the solution. From the equation we get:

$$P_T = P_f \frac{T}{T_f} \ .$$

In the case of weak aqueous solutions, the freezing-point of the solution may be considered, for this calculation, as practically the same as that of the solvent. Thus $T_f = 273°$ (the freezing-point of pure water), and T becomes $273 + t$, where t is the desired temperature in the Centigrade scale. Now the equation given above becomes:

$$P_T = P_f\left(1 + \frac{1}{273}t\right) = P_f(1 + 0.00367\,t) \ .$$

This is sufficiently accurate for dilute aqueous solutions.

The freezing-point method is the simplest and most satisfactory method for general use.

2. The boiling-point method: The boiling-point of a solution is always higher than that of the pure solvent, and its elevation is proportional to the osmotic pressure at that temperature. The relation between the two quantities for aqueous solutions is expressed as follows:

$$P_b = 43320\,\Delta_b \ ,^{1}$$

wherein P_b is the osmotic pressure in millimeters of mercury at the boiling-point of the solution, and Δ_b is the elevation of the boiling-point. The determination of the boiling-point of the solution and of distilled water is best made

[1] NERNST-PALMER, *Theoretical Chemistry* (London, 1895), p. 129. The pressure is again reduced to millimeters of mercury.

by Beckmann's improved apparatus for this purpose,[1] a description of which will be found along with that for the freezing-point determinations.

The correction for temperature may be made, as in the last case, by the application of the principle of Gay-Lussac directly, or by interpolation between P_b and P_f, the latter having been determined by the previous method.

The expression for the Gay-Lussac principle is of course the same, *mutatis mutandis*, as that given above:

$$P_T = P_b \frac{T}{T_b} ,$$

in which P_T is again the pressure in millimeters at the desired temperature T, in the absolute scale, and T_b is the absolute boiling-point of the solution. For this calculation the boiling-point of a weak aqueous solution may be considered the same as that of pure water. Thus $T_b = 373°$, the boiling-point of water, and $T = 273 + t$, where t is the desired temperature in the Centigrade scale. Making these changes in the above equation,

$$P_T = P_b \frac{273 + t}{373} .$$

The method of interpolation is expressed by the following equation:

$$P_t = P_f + \frac{t}{T_b - T_f}(P_b - P_f) ,$$

where P_t is the pressure at the desired temperature, t (Centigrade), and the other symbols are the same as above.

3. Method by observed vapor tension: As has already been stated, the vapor tension of the solvent is decreased by the presence of a solute. It is found that, for dilute solutions, this decrease in vapor tension is proportional to the

[1] E. BECKMANN, "Zur Praxis der Bestimmung von Molekulargewichten nach der Siedemethode," *Zeitschr. f. physik. Chem.*, Vol. VIII (1891), pp. 223–8.

osmotic pressure. This relation is expressed by the following equation:

$$P = \frac{\pi - \pi'}{\pi} \frac{0.0819\,T \times 1000\,s \times 760}{M} .$$ [1]

In this P is the osmotic pressure in millimeters at the absolute temperature T, π and π' are the vapor tensions observed at that temperature of the solvent and solutions respectively, s is the specific gravity of the solution, and M is the molecular weight of the pure solvent. In the case of dilute aqueous solutions, s may be put equal to unity (the specific gravity of the pure solvent instead of that of the solution), and M is 18 (the molecular weight of water). Making these substitutions in the above equation, we have:

$$P = \frac{\pi - \pi'}{\pi} \frac{0.0819\,T \times 1000 \times 760}{18} ,$$

or

$$P = \frac{\pi - \pi'}{\pi} 3458\,T .$$

The determination of the vapor tensions is best made by means of the method devised by Ostwald and Walker.[2] Two Liebig potash bulbs, one filled with the solution to be tested and the other with the pure solvent (the latter weighed), are joined in series and then attached to a weighed U-tube of pumice moistened with sulphuric acid. A slow current of air is passed, for six to twelve hours, through the series. The air first becomes saturated at the tension of the solution, and then, passing through the second bulb, becomes again saturated at the vapor tension of the pure solvent. A final weighing of the second bulb and of the

[1] NERNST-PALMER, *Theoretical Chemistry* (London, 1895), p. 126; also J. H. VAN'T HOFF, "Die Rolle des osmotischen Druckes in der Analogie zwischen Lösungen und Gasen," *Zeitschr. f. physik. Chem.*, Vol. I (1887), pp. 481-508.

[2] J. WALKER, "Ueber eine Methode der Bestimmung der Dampfspannung bei niederen Temperaturen," *Zeitschr. f. physik. Chem.*, Vol. II (1888), pp. 602-5; also OSTWALD-WALKER, *Manual of Physico-Chemical Measurements* (London, 1894), p. 188.

sulphuric-acid tube gives the required data. The amount of vapor removed from the two bulbs respectively is proportional to the vapor tensions of their contents. Thus if w denote the loss in weight in the second bulb and w' the gain in weight of the sulphuric acid,

$$\frac{w}{w'} = \frac{\pi - \pi'}{\pi} .$$

Therefore the equation given above may be written:

$$P = \frac{w}{w'} 3458\, T .$$

This method is difficult of operation and not very satisfactory. The whole apparatus must be surrounded by a jacket to keep all the parts at the same temperature; it is not necessary that the temperature be absolutely constant, however. The only advantage in this method over those previously described is that by this means the osmotic pressure can be determined for the temperature at which the solution is used, thus avoiding the correction for temperature.

II. CALCULATION OF OSMOTIC PRESSURE

a) When the pressure is produced by a non-electrolyte. —All solutions of non-electrolytes which contain the same number of molecules per unit volume of solution give the same osmotic pressure. From measurements made by Pfeffer we know that the osmotic pressure of a solution of sugar containing a gram-molecule per liter is the same as the gas pressure of a gram-molecule of gas occupying a liter volume. This pressure is 22.3 atmospheres, or 16,948 mm. of mercury, at 0° C., or 273° absolute. Thus, if we know the molecular weight of the solute and the number of grams per liter of solution, the calculation, on the principle that pressure varies as concentration, is simple enough. The correction for temperature is carried out by the principle of Gay-Lussac.

For mixed solutions of non-electrolytes the total osmotic pressure is the sum of the partial pressures due to the several solutes respectively.

b) When the pressure is produced by an electrolyte.— On account of the phenomena of ionization or dissociation, the calculation of the osmotic pressure of a solution of an electrolyte becomes somewhat complicated. The amount of ionization must be known in order to get the relative number of particles per unit volume. For instance, a gram-molecule of NaCl, in aqueous solution, occupying a liter volume, contains more particles than the same volume of a normal solution of sugar; some of the molecules have separated into Na and Cl ions.

The amount of ionization in any simple solution of an electrolyte can be determined by means of the method of electrolytic conductivity devised by Kohlrausch.[1] The conductivity is proportional to the number of free ions, and hence, knowing the conductivity both at the given concentration and at a concentration where ionization is complete, we can calculate the amount of ionization. The conductivity of many solutions has been determined by different authors in different units. Of course, all are reducible to C. G. S. units or to the conductivity of mercury, but it is immaterial for the present purpose what units are used, so long as the same ones are used throughout the same calculation. As the solution becomes more and more dilute, the conductivity approaches a limit. This limit is the conductivity at infinite dilution, where ionization is complete; it is usually denoted by λ_∞. Allow λ to denote the conductivity at the given concentration. Then $\frac{\lambda}{\lambda_\infty} = \alpha$, the fraction of the whole

[1] F. KOHLRAUSCH, *Leitfaden der praktischen Physik*, 7th ed. (Leipzig, 1892), p. 301; also F. KOHLRAUSCH UND L. HOLBORN, *Das Leitvermögen der Elektrolyte*, Leipzig, 1898; W. OSTWALD, "Ueber Apparate zur Bestimmung der Electrischen Leitfähigkeit von Electrolyten, *Zeitschr. f. physik. Chem.*, Vol. II (1888), pp. 561-67; OSTWALD-WALKER, *Manual of Physico-Chemical Measurements*, London, 1894.

number of molecules which are dissociated. Thus, if one out of every ten molecules were dissociated, a would equal $\frac{1}{10}$.

Now, if each molecule forms k ions, and if i denote the ratio of the actual osmotic pressure to that which would be obtained in the same concentration of a non-electrolyte solution, i may be found from the following:

$$i = 1 + (k - 1)a \ .$$

And if P denote the osmotic pressure developed in a solution of a non-electrolyte, of the same concentration as that whose pressure is to be found, P' being the required pressure, then

$$P' = Pi \ .$$

The conductivities of a great many solutions are to be obtained from published tables.[1] It is not necessary to give the methods for determining these conductivities here. They are thoroughly and completely discussed by Kohlrausch and Holborn. If the osmotic pressure is all that is required, and data for the conductivity of the given solute cannot be found in the published tables, then it is more expedient to determine the pressure by means of one of the indirect methods previously described than to determine the conductivity. If the proper concentration is not given in the tables, the conductivity for it is found by interpolation between the conductivities for the two concentrations nearest to it. If the table is rather extensive for the solution in question, so that conductivities for very low concentrations are given, it is usually safe to take the highest conductivity as λ_∞. If the table is not so complete, a limit for the conductivity has to be approximated from the trend of the given data. In using the published tables, it is very important that one bear in mind the difference between molecular and

1 W. C. D. WHETHAM, *Solution and Electrolysis* (Cambridge, 1895), pp. 218 ff.; also F. KOHLRAUSCH UND L. HOLBORN, *Das Leitvermögen der Elekrolyte*, Leipzig, 1898.

equivalent solutions. Most of the tables consider as a standard solutions containing a gram-equivalent per liter. These are easily transformed into gram-molecular solutions by dividing the given concentration by the number representing the valency of the compound. Thus one-tenth gram-equivalent per liter of Na_2SO_4 is identical with one-twentieth gram-molecular solution of the same salt.

For very weak solutions of mixed electrolytes the above method may be resorted to. But for solutions of mixed electrolytes and non-electrolytes, and for strong solutions of electrolytes, no method of calculation has yet been discovered. The only practical way open in such a case is to resort to the methods of freezing- and boiling-points. It is often best to make use of both these methods, and to interpolate between them for the normal temperature, inasmuch as ionization often increases rapidly at higher temperatures. Of course, where chemical reaction occurs between the different solutes, the osmotic pressure of the solution will not be constant until chemical equilibrium has been attained.

PART II

PHYSIOLOGICAL CONSIDERATIONS

INTRODUCTION

So IMPORTANT a part do diffusion and osmotic pressure seem to play in the vital processes of plants, that it is well-nigh impossible to consider any phase of vegetable physiology without some reference to these subjects. It is obviously not to the point, however, to attempt here a discussion of every phenomenon in plant life into which they enter. Rather will attention be directed to certain groups of phenomena wherein diffusion and osmotic pressure seem to be fundamental factors. Thus, it is hoped, may be formed a general conception of the trend which modern study is taking along these lines.

Of the four following chapters, the first three have to do with osmotic pressure as an *internal* factor in the life of the plant; in them are considered the most important effects of the development of diffusion tensions within the plant body. In the last chapter are brought together the responses of the organism to variations in the osmotic pressure of the surrounding medium. Such division of the subject is merely expedient; it is purely artificial, for the organism and its surrounding medium are physically almost as truly continuous as are a mass of ice and the water in which it floats. Also—a fact which is often apparently lost sight of—every portion of the plant body is a portion of the environment of every other portion. This is of fundamental importance, especially in the physiology of multicellular forms. However, the plant body is a fairly definite thing, and in the present state of our knowledge the above classification of environmental factors is perhaps as good as any other.

In the following pages authors are cited for the most

important pieces of research, mainly for the more recent ones. References are not given for material which may be regarded as a matter of common knowledge. To those who wish full citations for the period up to the time of its publication, Ewart's admirable translation of Pfeffer's *Physiology of Plants* will be found of great service.

CHAPTER I

TURGIDITY

I. PROTOPLASM AND ITS LIMITING MEMBRANES

ANYTHING resembling an exact knowledge of the nature of protoplasm is very remote from us as yet, but we may be fairly certain of this, at least, that, whatever else it may be, the vital substance is a mixture of many soluble colloids dissolved in, or impregnated with, an aqueous solution of many different crystalloids. Colloids are very inactive as far as diffusion and osmotic pressure are concerned. Thus, if an internal diffusion tension is developed within a mass of protoplasm, it must be mainly due to the crystalloids dissolved in the contained water. On this account it must come about that a mass of colloid substance inclosing within its body an osmotic solution, and surrounded by another osmotic solution, will act somewhat as though the former solution were surrounded by a semi-permeable membrane. Because of their slow rate of diffusion, colloid particles must in a measure block the way for the diffusion of crystalloid particles. Hence, if the more concentrated osmotic solution be within the colloid mass, there will be developed a slight osmotic pressure within the mass, which will hasten its normal process of swelling by imbibition. With no truly semi-permeable membrane about it, no state of equilibrium can be attained between a colloid body and the surrounding medium, until, by the slow outward diffusion of the crystalloid particles and by the entrance of water, there comes about a uniform concentration both within and without.

In the author's experiments with gelatin plate cultures of Stigeoclonium the following observations were made, which

appear to have a bearing in this connection: A somewhat concentrated solution of mineral salts was thickened by the addition of enough gelatin to make a firm mass at ordinary temperatures. On the surface of this mass were placed single drops of a dilute solution having the same chemical nature as the one contained within the gelatin plate, and the whole was kept in a moist chamber. After four or five hours it was always noted that the drops of liquid had disappeared; they had been absorbed into the colloid mass. If, however, the more dilute solution were contained within the gelatin plate, and drops of a concentrated solution were placed upon its surface, it took very much longer for total absorption to occur. For the first few hours there was usually even an observable increase in the size of the liquid drops. Eventually absorption occurred, but it was often at the end of a period of more than twenty-four hours. Of course, if there had been a semi-permeable membrane between the drops and the gelatin, absorption would not have taken place. The gelatin mass is not semi-permeable, but seems merely to retard the process of diffusion of crystalloid solutes.

If a mass of such gelatin, containing a strong osmotic solution and surrounded by a semi-permeable membrane, be placed in water or a weaker solution, this membrane will be stretched by the internal pressure practically as though no colloid were present, and a state of equilibrium will be reached only when the resilience of the membrane equals the osmotic pressure within. This has been demonstrated experimentally by Traube and Pfeffer.[1] In such a case the osmotic pressure of the colloid is of such an order as to be negligible.

Now, any mass of protoplasm is very much the same sort of a colloid mass as the gelatin just described. Its outer

[1] M. TRAUBE, "Experimente zur Theorie der Zellenbildung und Endosmose," *Arch. f. Anat. u. Physiol.*, Physiol. Abth., Jahrg. 1867, pp. 87-165. Also PFEFFER-EWART, *Physiology of Plants*, Cambridge, 1900, p. 106.

layer is so transformed (perhaps in many instances by mere contact with the external solution and with surrounding objects) that it is almost perfectly impermeable to many solutes, but remains permeable to water. The protoplast of every normal vegetable cell is thus surrounded by a more or less perfectly semi-permeable layer, the ectoplast. If the ectoplast is ruptured in any way, it is soon re-formed, unless disorganization of the protoplast ensues.[1] In such cases (*e. g.*, in Myxomycete plasmodia, etc.), where unmodified protoplasm is brought into contact with surrounding medium, it is perhaps partially on account of its colloidal nature that the contained crystalloids are not immediately lost by diffusion, instead of being retained, as they are, until a new surface layer can be formed.

The internal osmotic pressure, which results when the inclosed solution is more concentrated than the external one, tends to stretch the surface layer and enlarge the protoplast. Against this pressure is brought to bear whatever cohesive and resilient force the ectoplast may possess; but this, from the semi-fluid nature of protoplasm itself, must be of a low order. In naked cells this fact prevents the internal pressure from ever becoming very great; in such cases rupture and destruction of the protoplasm would inevitably result. But if the protoplasm is surrounded by a cellulose membrane, as in the case of the majority of plant cells, this condition is entirely altered; the swelling of the protoplasmic mass is checked at the limit of extensibility of the inclosing cellulose layer. Pressure upon the ectoplast is transmitted immediately to the cell wall, and the latter is stretched according to its extensibility and to the pressure applied. In the condition of strain resulting from the interaction of the force of osmotic pressure (the diffusion

[1] W. PFEFFER, "Zur Kenntniss der Plasmahaut u. d. Vacuolen," etc., *Abhandl. d. k. sächs. Ges. d. Wiss. zu Leipzig*, math.-physik. Klasse, Vol. XVI (1890), pp. 187–344.

tension of the solute particles) on the one hand, and that of resilience of the cellulose membrane on the other, a rigidity and firmness of the cell as a whole is brought about, just as a football or bicycle tire becomes rigid and firm upon being inflated with gas. This rigidity is termed turgescence, or turgidity. The term "turgor" has also been applied to this condition, but it is better to reserve this word to express the osmotic pressure of the internal fluid.[1] In order that the protoplast may retain its osmotic properties, the cellulose wall must be permeated with water. This is absolutely essential for the development of turgidity, since osmotic pressure is not active beyond the limits of the solvent. It is, indeed, true that the cellulose envelope of every active cell is saturated with water.

Thus far only those cells have been considered which are completely filled with protoplasm. This is the condition in young cells, but mature cells are not usually so filled; as growth progresses, vacuoles of a watery fluid appear in the protoplasm. These increase in size and fuse together, until at length there is a single large vacuole within the protoplasmic mass. The typical cell of plant tissues consists of a cellulose wall lined internally by a layer of protoplasm, which incloses a mass of aqueous solution, the cell sap, containing sugars and various other solutes. The lining layer of protoplasm is bounded externally, where it comes in contact with the cell wall, by the ectoplast. Internally, toward the vacuole, it is bounded by a similar membrane, the tonoplast. The cellulose wall is readily permeable to water and solutes, but the protoplasmic lining, with its two somewhat differentiated limiting layers, normally acts like a semi-permeable membrane, allowing water to pass quite freely, but hindering, and often seeming absolutely to prevent, the passage of solutes.

[1] *Cf.* E. B. COPELAND, "The Mechanism of Stromata," *Ann. Bot.*, Vol. XVI (1902), p. 330; IDEM, "The Rise of the Transpiration Stream," *Bot. Gaz.*, Vol. XXXIV (1902), p. 173.

It is in these vacuolated cells that turgidity is developed to its greatest extent. It may be that continued concentration of the solution within the protoplasm itself may soon reach a limit beyond which it cannot go without affecting those energy transformations which we term vital activity or life. An alteration in the activities of the protoplasm thus produced may result in a change in its permeability in one way or another. And changes of this sort accompanied by changes in the chemical activity within the protoplast may account for the formation of the vacuole and the secretion of osmotically active materials into it. It was shown by Loeb[1] that changes in the concentration of different ions in the protoplasm of animal muscle bring about marked changes in its power of absorbing water.

At any rate, however the vacuole may arise, the turgidity of the normal mature plant cell is mainly due to the osmotic pressure of the cell sap and to the semi-permeability of the surrounding protoplasmic layer. The part played in the development of turgidity by the tonoplast and ectoplast and by the unmodified protoplasm itself, has not been worked out. Indeed, the semi-permeability of this layer can perhaps be attained only through the co-operation of the three somewhat distinct layers which make up the lining of the cellulose wall. Although De Vries[2] was able to separate the tonoplast from the remainder of the protoplasmic mass, yet it soon lost its peculiar properties when the surrounding protoplasm was killed. Pfeffer[3] has shown that the tonoplast and ectoplast are equivalent and are probably formed in the

[1] J. LOEB, "On Ion Proteid Compounds and Their Rôle in the Mechanics of Life Phenomena"; I, "The Poisonous Character of a Pure NaCl Solution," *Am. Jour. Physiol.*, Vol. III (1900), pp. 327–38.

[2] HUGO DE VRIES, "Plasmolytische Studien über die Wand der Vacuolen," *Jahrb. f. wiss. Bot.*, Vol. XVI (1885), pp. 465–598. The tonoplast is not a special cell organ, as De Vries was led to suppose.

[3] W. PFEFFER, "Zur Kenntniss d. Plasmahaut u. d. Vacuolen," etc., *Abhandl. d. k. sächs. Ges. d. Wiss. zu Leipzig*, math.-physik. Klasse, Vol. XVI (1890), pp. 187–344.

same manner. In consideration of such facts as these much stability cannot be predicated of these membranes, and thus, in a discussion of the osmotic properties of the cell, it will probably be safer to regard the intra-vacuolar pressure as arising from the semi-permeability of the lining layer of protoplasm as a whole.

In a vacuolated cell the osmotic pressure sometimes becomes so great as to burst the cellulose membrane. This is notably so in the bursting of the asci in certain ascomycetous fungi and in the explosion of the hypo-sporangial region in Pilobolus. Many plant cells may be made to burst in this way by immersing them in a very weak solution or in distilled water. For example, Lidforss[1] found that the pollen grains of many plants (notably the Liliaceae, as Funkia, Asphodelus, Anthericum, etc.) exploded in this way when put into water. Noll[2] has shown that when certain marine Siphoneae are placed in pure water their filaments are apt to burst. Also Curtis[3] noted that when the common molds were placed in water after having been accustomed to a concentrated solution, the hyphal tips often burst in the same manner. Similar observations were made among animals by Gogorza,[4] who records the bursting of blood corpuscles in certain marine forms when they were killed by being placed in fresh water.

II. PLASMOLYSIS

When a plant cell is surrounded by a solution of greater concentration than that contained within its vacuole, the phenomenon of plasmolysis occurs. The greater osmotic

[1] B. LIDFORSS, "Zur Biologie des Pollens," *Jahrb. f. wiss. Bot.*, Vol. XXIX (1896), pp. 1–138.

[2] F. NOLL, "Beitrag zur Kenntniss der physikalischen Vorgänge welche den Reizkrümungen zu Grunde liegen," *Arb. d. bot. Inst. in Würzburg*, Vol. III (1888), p. 496.

[3] C. CURTIS, "Turgidity in Mycelia," *Bull. Torr. Bot. Club*, Vol. XXVII (1900), pp. 1–13.

[4] GOGORZA Y GONZALEZ, "Influencia del aqua dulce en los animales marinos," *Annales de la soc. espagn. hist. nat.*, Vol. XX (1891), pp. 220–71.

pressure of the solutes outside, together with the slight resilience of the protoplasmic layer, cause a contraction of the protoplasm resulting in its separation from the inclosing cellulose wall. If the process of plasmolysis is complete the vacuole may disappear, practically all the water passing out. In such cases the protoplasm often takes on the form of a solid sphere, which lies near the middle of the cell or at one side. Plasmolysis comes about within a very few minutes after the cell has been placed in the plasmolyzing solution. It is partly from the latter fact that the cellulose wall is known to be permeable to solutes as well as to water. If it were not so, either plasmolysis would not occur, or the cellulose membrane would follow the protoplasm in its withdrawal toward the center of the cell. The cellulose wall does, indeed, contract to a certain measurable extent, but this is due entirely to its elasticity; it simply returns to its normal state of equilibrium when the internal pressure of the turgid protoplasmic sac is removed.

This fact of plasmolysis has long been known,[1] but the true interpretation of it was due to De Vries[2] and Pfeffer.[3] After the relation which exists between plasmolysis, turgidity, and osmotic pressure was once established, it was De Vries[4] who pointed out that in the former of these phenomena we possess a means of measuring the amount of osmotic pressure in any given cell. His method has been used very largely in such measurements. It may be described as follows: If a piece of plant tissue be placed in a concentrated solution

[1] For a historical treatment of this subject see W. PFEFFER, "Zur Kenntniss der Plasmahaut u. d. Vacuolen," etc., *Abhandl. d. k. sächs. Ges. d. Wiss. zu Leipzig*, math.-physik. Klasse, Vol. XVI (1890), p. 316.

[2] H. DE VRIES, *Untersuchungen über die mechanischen Ursachen der Zellstreckung*, Leipzig, 1877.

[3] W. PFEFFER, *Osmotische Untersuchungen*, Leipzig, 1877, pp. 121 ff.

[4] H. DE VRIES, "Eine Methode zur Analyse der Turgorkraft," *Jahrb. f. wiss. Bot.*, Vol. XIV (1884), pp. 427–601. IDEM, "Osmotische Versuche mit lebenden Membranen," *Zeitschr. f. physik. Chem.*, Vol. II (1888), pp. 415–32; IDEM, "Isotonische Koeffizienten einiger Salze," *ibid.*, Vol. III (1889), pp. 103–12.

of potassium nitrate, plasmolysis will occur. If tissues with colored cell sap, such as portions of the lower epidermis of the leaves of Tradescantia, are used, contraction of the vacuole may be seen very readily under the microscope. The coloring matter of the sap fails to pass the protoplasmic layer, and thus plasmolysis is accompanied by a deepening of the color of the sap. If the experiment be repeated on fresh bits of tissue, continually weaker and weaker solutions of potassium nitrate being used, a concentration of the latter will at length be reached, such that no plasmolysis will occur. But plasmolysis indicates that the external solution is more concentrated than that within the vacuole, and its failure to appear indicates that the cell sap is more concentrated than the external solution. Therefore, it may be considered that the maximum concentration of potassium nitrate which does not cause plasmolysis is isosmotic (*i.e.*, has the same osmotic pressure) with the cell sap. If we can choose a plasmolyzing substance to which the protoplasmic membrane is very nearly or quite impermeable[1] (see the following section), this will give a very exact method for measuring turgor pressure. In this way De Vries was able to show that, in general, the concentration causing plasmolysis was always the same, no matter what substance was used to produce it. There were some exceptions, however, glycerin being the most notable of those used by him. He found, too, that certain electrolytes gave extraordinarily high osmotic pressures. The last is now known to be due to ionization. The "isotonic coefficients" given by this author express approximately the amount of ionization for the concentrations which he used. The results are exceedingly valuable, for they have led to great advance, not only in physiology, but also in physical chemistry; but since these

[1] DE VRIES has given some very pointed directions for the critical use of his method in "Zur plasmolytischen Methodik," *Bot. Zeitg.*, Vol. XLII (1884), pp. 289-98.

coefficients hold true only within certain limits, and since other more accurate methods are now available for determining the amount of ionization, a discussion of them is here omitted.[1]

On the animal side the method of plasmolysis has been used by Hamburger and others[2] for determining the osmotic pressures of the fluid contained in blood corpuscles.

Another method for comparing the osmotic pressure of the fluid contained in red blood corpuscles with that of the surrounding fluid was devised by Köppe,[3] and further used by Löb[4] and Hedin.[5] The total volume of all the corpuscles of a given amount of blood was first determined by separating them from the plasma by means of the centrifuge. Then a known amount of blood was added to a given volume of salt solution of known concentration, and the mixture was shaken thoroughly. The corpuscles were then separated from the solution on the centrifuge, and their total volume carefully measured. If the resulting volume was less than the normal for the given amount of blood, the conclusion

[1] It will be well for physiology when the practical use of these coefficients dies out entirely.

[2] H. J. HAMBURGER, "Ueber den Einfluss chemischer Verbindungen auf Blutkörperchen in Zusammenhang mit ihren Molekulargewichten," *Arch. f. Anat. u. Physiol.*, Physiol. Abth., Jahrg. 1886, pp. 476–87; IDEM, "Ueber die durch Salz- und Rohrzuckerlösungen bewirkten Veränderungen der Blutkörperchen," *ibid.*, Jahrg. 1887, pp. 31–47; IDEM, "Die Permeabilität der rothen Blutkörperchen in Zusammenhang mit den isotonischen Coefficienten," *Zeitschr. f. Biol.*, Vol. XXVI (1889), pp. 414–33; G. GRYNS, "Ueber d. Einfluss gelöster Stoffe auf d. rothen Blutzellen, in Verbindung mit d. Erscheinungen der Osmose u. Diffusion," *Pflügers Arch. f. d. ges. Physiol.*, Vol. LXIII (1896), pp. 86–119; H. KÖPPE, "Physiologische Kochsalzlösung—Isotonie—osmotischer Druck," *ibid.*, Vol. LXV (1897), pp. 492–502.

[3] H. KÖPPE, "Eine neue Methode zur Bestimmung isotonischer Konzentrationen," *Zeitschr. f. physik. Chem.*, Vol. XVI (1895), pp. 261–88.

[4] W. LÖB, "Ueber Molekulargewichtsbestimmung von in Wasser löslichen Substanzen mittels der rothen Blutkörperchen," *ibid.*, Vol. XIV (1894), pp. 424–32.

[5] S. G. HEDIN, "Ueber d. Brauchbarkeit der Centrifugalkraft für quantitative Blutuntersuchungen," *Pflügers Arch. f. d. ges. Physiol.*, Vol. LX (1895), pp. 360–404; IDEM, "Ueber d. Bestimmung isotonischer Konzentrationen durch Zentrifugieren von Blutmischungen," *Zeitschr. f. physik. Chem.*, Vol. XVII (1895), pp. 164–70; IDEM, "Einige Bemerkungen Köppes Abhandlung: Ueber eine neue Methode, etc.," *ibid.*, Vol. XXI (1896), pp. 272–6.

was drawn that the corpuscles had lost water, and hence that the surrounding solution was of higher osmotic pressure than the internal one. Several slight modifications of the method were used, and many different solutions were compared, the results being quite uniform with those obtained by direct observation of the cells by De Vries and Hamburger.

Still another manner of comparing osmotic pressures of various solutions by means of plasmolytic phenomena in living cells is that used by Wladimiroff,[1] who brought motile bacteria into requisition for the purpose. He found that these organisms cease to be motile when the osmotic pressure of the surrounding fluid attained a certain magnitude. Using as a criterion the degree of concentration at which motion ceased, he compared the osmotic pressures of a number of solutions. His results are, in general, uniform with those of the other authors just mentioned. The loss of motion was due to extraction of water in a manner exactly analogous to plasmolysis.

In making turgor determinations by the plasmolytic method the results may be given in various ways. The usual method has been to give them in terms of a per cent. solution of potassium nitrate, sometimes of sodium chlorid, sometimes of sugar, etc. But with this method, whenever it is desired to compare pressures which have been measured by means of different plasmolyzing solutions, it becomes necessary to make calculations which involve the molecular weights of the substances used. A much better way to express turgor pressures is in terms of fractions (*e. g.*, tenths) of a molecular solution. But this, although it suffices for non-electrolytes, fails utterly for electrolytes, because of the unequal dissociation of different compounds. A $\frac{1}{10}$ gram-molecular solution of KNO_3 will give a much greater osmotic

[1] A. WLADIMIROFF, "Osmotische Versuche an lebenden Bakterien," *Zeitschr. f. physik. Chem.*, Vol. VII (1891), pp. 529–43; also *Zeitschr. f. Hygien*, Vol. X (1891), pp. 89–110.

pressure than a $\frac{1}{10}$ gram-molecular solution of glucose. A method must therefore be devised which will render it possible to compare readily the osmotic pressures of electrolytes and non-electrolytes. This can be done by means of any unit of pressure. The mercury column may be used, or large pressures may be expressed in atmospheres. Recently Errera[1] has suggested a special unit for measuring osmotic pressure, which he proposes to call the *tonie*. It is to be equal to the pressure of one dyne upon a surface of one square centimeter. For larger measurements he suggests the term *myriotonie*, equal to ten thousand *tonies*. It is difficult to see how this new unit possesses any advantage over the mercury unit for practical work. For plasmolytic purposes it is much more convenient to reduce all measurements to terms of a molecular solution of a non-electrolyte. Thus comparison becomes easy and the absolute pressure per unit surface can be readily found from the relation

$$M = 22.3 \text{ atmospheres, or } 1695 \text{ cm. Hg,}$$

where M is the osmotic pressure of a molecular solution of a non-electrolyte. Of course, in making up solutions of an electrolyte for use by this method it must be borne in mind that the desideratum is not a molecular solution of the electrolyte, but *a solution whose osmotic pressure will just equal that of a given solution of a non-electrolyte.* Thus a solution of NaCl whose osmotic pressure is, say, $\frac{2}{10}$ M, must be considerably more dilute than a $\frac{2}{10}$ molecular solution of that salt.

III. THE PERMEABILITY OF THE PROTOPLASMIC LAYERS

The often repeated statement that the protoplasmic layer is not permeable to solutes needs to be modified as follows: To some substances it is probably absolutely impermeable

[1] L. ERRERA, "Sur la myriotonie comme unité dans les mesures osmotiques," Extr. des *Bull. de l'acad. roy. de Belgique*, Vol. III (1901), pp. 135-53.

under certain conditions; to the majority of substances it is usually very slightly permeable, but under certain conditions its permeability may increase; and to some substances it is usually very readily permeable. Further than this, the protoplasm of different plants, and even of different cells in the same plant, has different osmotic properties. The condition of things is thus seen to be very complex. It will be of value to pass in review the most important fragments of evidence which have been accumulated upon this question of protoplasmic permeability.

a) *Test by the plasmolytic method.*— There are several ways of testing the permeability of the protoplasmic sac. The one most frequently resorted to is that of plasmolysis. A bit of tissue or a unicellular organism is subjected to the osmotic action of solutions of the substance which is to be tested, these being of several different concentrations. If plasmolysis occurs in a solution of rather high concentration, this fact is taken as evidence that the protoplasm of the given cells is either impermeable to the solute or very slightly permeable. Of course, it is also theoretically possible that in this case the substance used penetrates the protoplast to some extent and causes a polymerization or precipitation of the osmotically active solutes within the sap. There is no evidence for this phenomenon, however, and its general improbability throws it out of the category of serious objections to the plasmolytic method. If, after being left a short time in the plasmolyzing solution, the cells regain their normal condition, it shows either that the protoplasm is somewhat slowly penetrated, or else that some osmotic material has been secreted within the cell. If plasmolysis occurs at a very low concentration, it is sufficient proof that the substance enters the protoplasm; for such plasmolysis is due to alteration in the membrane through poisonous action, or to a precipitation or some similar change

within the vacuole, either of which phenomena could not take place without penetration. If plasmolysis does not occur even at high concentrations, we have evidence that the protoplasmic sac is not only penetrable to the substance used, but that this substance has no marked immediate toxic action.

Cane sugar, glucose, KNO_3, and NaCl are usually found to produce permanent plasmolysis. Plant cells placed in concentrated solutions of these substances do not, as a rule, regain their original turgid condition as long as they remain therein ; no perceptible penetration occurs. However, there are many cells whose protoplasts are more or less permeable to these compounds, and there are all gradations between absolute impermeability and rather slow permeability. One extreme of this series is Massart's Bacterium termo,[1] which was not plasmolyzed at all in strong solutions of cane sugar and KNO_3.

But in most cases plasmolysis is the first result of irrigating the cells with the test solution, and it is only after the lapse of some time that the first effect disappears. The gradual inward diffusion of the external osmotic substance, or, in some cases, the gradual secretion of an osmotic substance within the cells, finally brings about an equalization of the internal and external pressures. Then the original internal pressure, produced by the solutes within the vacuole, becomes again effective in producing turgidity. De Vries[2] found that the tonoplasts of various plant cells were generally freely penetrated by acids and alkalies, but that salts passed these membranes much more slowly. However, many cells were found which, after being plasmolyzed in a solution of KNO_3 or NaCl, gradually returned to their origi-

1 MASSART, "Sensibilité et adaption des organismes à la concentration des solutions salines," *Arch. de biol.*, Vol. IX (1899), pp. 515–70.

2 H. DE VRIES, "Plasmolytische Studien über die Wand der Vacuolen," *Jahrb. f. wiss. Bot.*, Vol XVI (1885), pp. 465–598.

nal condition if left in the plasmolyzing solution. This author also observed that the presence of an acid or base, or of any other poisonous substance, made the protoplasm rapidly permeable to such salts as KNO_3 and NaCl. The cells of the epidermis of leaves of Tradeacantia, Curcuma, and Begonia rex appeared to be impermeable to KNO_3. The same author[1] found the protoplasm of beets to be permeable to NaCl. Janse[2] found a similar return of turgidity in the case of marine algæ (*e. g.*, Chætomorpha) which were allowed to remain in a solution of KNO_3 or of NaCl which plasmolyzed them at first. He also found that the protoplasts of these algæ are permeable to cane sugar. When plasmolysis was brought about in a solution of this substance, turgor gradually returned, but this process took about four times as long here as in a KNO_3 solution. In Spirogyra the same general facts were observed, but the permeability is not as marked here as in the marine forms. In summing up the results of his second paper, this author states that he has found the protoplasm of the following five plants permeable as follows:

Chætomorpha is permeable to KNO_3, NaCl, cane sugar.
Spirogyra is permeable to KNO_3, NaCl, grape sugar.
Tradescantia and Curcuma are permeable to KNO_3, NaCl.
Stratiotes is permeable to KNO_3.

Glycerin and urea have been shown by De Vries[3] and Klebs[4] to penetrate nearly all plant cells with great readi-

[1] H. DE VRIES, "Sur la perméabilité du protoplasma des betteraves rouges," *Arch. néerl.*, Vol. VI (1871), pp. 117–26.

[2] J. M. JANSE, "Plasmolytische Versuche an Algen," *Bot. Centralbl.*, Vol. XXXII (1887), pp. 21–6; IDEM, "Die Permeabilität des Protoplasma," *Verslag. en Mededeel. d. k. Akad. v. Wetensch. te Amsterdam*, 3 Reihe, Vol. IV (1888), p. 332.

[3] H. DE VRIES, "Ueber den isotonischen Coefficient des Glycerins," *Bot. Zeitg.*, Vol. XLVI (1888), pp. 229 ff.; IDEM, "Ueber die Permeabilität der Protoplaste für Harnstoff," *ibid.*, Vol. XLVII (1889), pp. 309 ff.

[4] G. KLEBS, "Beiträge zur Physiologie der Pflanzenzelle," *Unters. aus d. bot. Inst. zu Tübingen*, Vol. II (1888), pp. 489 ff.; IDEM, "Beiträge zur Physiologie der Pflanzenzelle," *Ber. d. deutsch. bot. Ges.*, Vol. V (1887), pp. 181–9.

ness. Plasmolysis occurs, but is of short duration ; Overton[1] found that it took from two to five hours for equilibrium to be re-established in solutions of these substances.

There are exceptions here also, however, for De Vries found that the cells of the bud scales of Begonia manicata were almost impermeable to glycerin and urea. Jennings[2] states that paramoecia are permanently plasmolyzed in glycerin.

By an extensive investigation of the plasmolytic behavior of various organic compounds Overton[3] found that a great number of these do not produce plasmolysis at all, so rapidly do they penetrate the protoplasm. Among these substances may be named : ethyl alcohol, ethyl ether, formaldehyde, chloral hydrate, acetone, methyl cyanid, furfurol, caffein, etc. The list includes practically all of the aliphatic alcohols and related compounds which are soluble in water, and also a number of soluble aromatic compounds, such as anilin, acetanilid, phenol, phloroglucin, etc. Although the author does not express himself on this point, it seems probable that the apparent plasmolysis which occurs when plant cells are placed in strong alcohol is due, not to osmotic pressure, but to an increased permeability in the osmotic membranes (due to the poison) and also to an active contraction on the part of the protoplasm. The same author shows that there are all gradations in rapidity of penetration, from those substances which fail to plasmolyze at all to those which produce permanent plasmolysis. In all these cases he found that the protoplasmic sac is as readily permeated outward as inward. Practically all liquids which

[1] E. OVERTON, "Ueber die osmotischen Eigenschaften der lebenden Pflanzen- und Tierzelle," *Vierteljahrschr. d. Naturf.-Ges. in Zürich*, Vol. XL (1895), pp. 159–84.

[2] H. S. JENNINGS, "Studies on the Reactions to Stimuli in Unicellular Organisms": I, "Reactions to Chemical, Osmotic, and Mechanical Stimuli in the Ciliate Infusoria," *Jour. Physiol.*, Vol. XXI (1897), pp. 258–322.

[3] E. OVERTON, *loc. cit.* The table of compounds occurs on p. 181.

are soluble in water penetrate readily, glycerin being one of the slowest. It appears as if the power to penetrate decreased with increasing specific gravity. Overton finds that the same thing is generally true of animal cells also, and—what is still more striking—that the amount of alcohols, etc., which plant and animal cells are able to bear is nearly the same.

When the solute fails to penetrate the protoplast, the osmotic concentration necessary for plasmolysis is constant, no matter what the solute may be. But the more readily it penetrates, the higher the concentration necessary to bring about plasmolysis, until at last, as in the alcohols and ethers, this phenomenon does not truly occur at all.

b) *Direct test of penetrability.*—Another method of determining the extent of permeability manifested by protoplasm is to identify the diffusing substance after it has passed the plasmic layer. De Vries[1] showed that the penetration of dilute ammonia into the cells of the red beet can be demonstrated by the reaction of the colored cell sap to this substance. The red sap changes to blue upon contact with an alkali. By choosing other cells whose sap contains red and blue dissolved pigments, Pfeffer[2] showed that not only ammonia, but also the caustic alkalies and alkaline carbonates as well as acids (such as tartaric, phosphoric, and carbonic) pass very rapidly through plant protoplasm. We may consider that this at least proves that the H and OH ions penetrate. In some cases a precipitate may be produced within the vacuole by the reaction of the penetrating substance with the materials of the cell sap. This is the case with caffein, antipyrin, and some others.[3]

[1] H. DE VRIES, "Sur la perméabilité du protoplasme des betteraves rouges," *Arch. néerl.*, Vol. VI (1871), p. 124; IDEM, "Sur la mort des cellules végétales," *ibid.* (1871), pp. 245-95.

[2] W. PFEFFER, *Osmotische Untersuchungen*, Leipzig, 1877, p. 140.

[3] PFEFFER-EWART, *Physiology of Plants*, 1900, p. 98.

Another manner of carrying out the direct test is to place cells in the solution to be tested and, after sufficient time has elapsed, to treat them with a reagent which will penetrate and also give a visible reaction with the substance to be tested. Thus, if penetration took place in the first solution a microchemical test for the solute should be obtained within the vacuole. In this way diphenylamin was first used by Molisch[1] to detect KNO_3 in plant cells. With this reagent a nitrate is indicated by the appearance of a blue color. It appears from the work of Molisch, Wieler, De Vries, and Janse, in which this method was used, that plant protoplasm is very generally penetrated by KNO_3 in dilute solution. In a similar manner tests have been made with Fehling's solution, which prove the penetration of glucose.

Janse[2] showed in this manner that Spirogyra protoplasts are penetrated by KNO_3. Wieler[3] worked with entire plants of the angiosperm group and obtained similar results. By means of diphenylamin and sulphuric acid he was able to demonstrate that NO_3 ions penetrate the protoplasts of seedling beans, sunflowers, etc. By platinum chlorid he also demonstrated the penetration of K ions. Stems placed in a sugar solution formed starch, while a control without sugar failed to do so. It made no difference whether cane sugar or glucose were used. This proves the power of these sugars to penetrate the protoplasts in stems. The last-named author also presents evidence that the roots of seedlings of Vicia faba are able to absorb glucose from a solution. Of

[1] H. MOLISCH, "Ueber den mikrochemischen Nachweiss von Nitraten und Nitriten in der Pflanze mittelst Diphenylamin oder Brucin," *Ber. d. deutsch. bot. Ges.*, Vol. I (1883), pp. 150-55; also *Sitzungsber. d. kais. Akad. d. Wiss. zu Wien*, math.-nat. hist. Klasse, Vol. I (1887), p. 221.

[2] J. M. JANSE, "Plasmolytische Versuche an Algen," *Bot. Centralbl.*, Vol. XXXII (1887), pp. 21-6; IDEM, "Die Permeabilität des Protoplasma," *Verslag. en Mededeel. d. k. Akad. v. Wetensch. te Amsterdam*, 3 Reihe, Vol. IV (1888), p. 332.

[3] A. WIELER, "Plasmolytische Versuche mit unverletzten Phanerogamen," *Ber. d. deutsch. bot. Ges.*, Vol. V (1887), pp. 375-80.

course it is possible that, in these cases, the substance in question does not pass the protoplasm as such, but is modified at the surface of the ectoplast and penetrates in another form. For this question there seems to be as yet no method of attack. Wortmann[1] believed he had evidence that the starch in the endosperm of seeds was not acted upon by an enzyme but by the protoplasm itself; this, however, has been disproved.[2] Since protoplasm contains so many enzymes of one sort and another it seems impossible to gather evidence as to whether a given action takes place within the protoplasmic mass or outside of it. It is probable that it occurs wherever the enzymes are present, whether this be within or without.

The penetration of anilin dyes has been studied extensively by Pfeffer,[3] who showed, for example, that the sap of living cells may be strongly stained by the inward diffusion of methyl blue, methyl violet, etc. Certain anilin dyes may be absorbed into the living protoplasm itself and held there so as to give a marked stain. Even the nucleus may be so stained while living by means of dahlia, mauvein, etc.[4]

In most of these experiments there is an accumulation of the stain in the vacuole or within the protoplasm. Thus, if plants of Elodea canadensis be placed for several days in a weak solution of methyl blue, they become visibly stained, while the external solution loses its color. Examination shows that the protoplasm itself is not colored, but that the

[1] J. WORTMANN, "Ueber den Nachweiss, das Vorkommen und die Bedeutung des diastatischen Enzyms in den Pflanzen," *Bot. Zeitg.*, Vol. XLVIII (1890), pp. 581 ff.

[2] B. HANSTEEN, "Ueber die Ursachen der Entleerung der Reservestoffe aus Samen," *Flora*, Vol. LXXIX (1894), pp. 419–29.

[3] PFEFFER-EWART, *Physiology of Plants*, Cambridge, 1900, p. 96. References to an extensive literature are there given, the most important of which is: W. PFEFFER, "Ueber Aufnahme von Anilinfarben in lebenden Zellen," *Unters. aus d. bot. Inst. zu Tübingen*, Vol. II (1886), pp. 179–331; see also E. OVERTON, "Studien über d. Aufnahme der Anilinfarben durch die lebende Zelle," *Jahrb. f. wiss. Bot.*, Vol. XXXIV (1900), pp. 669–701.

[4] D. H. CAMPBELL, "The Staining of Living Nuclei," *Unters. aus d. bot. Inst. zu Tübingen*, Vol. II (1888), pp. 569–81.

dye has accumulated in the vacuole, there becoming much more concentrated than was the original external solution. This phenomenon will be discussed under *e*).

Another method by which direct determinations of permeability may be made is to analyze the plant or its juice after the culture has been grown some time in a medium of known content. In this way von Mayenburg[1] found that, out of a series of substances used in his culture fluids for Aspergillus niger, only glycerin was absorbed in sufficient quantity to be worthy of consideration as an osmotically active solute within the cells.

c) *Absorption test.*— If the concentration of the surrounding medium is carefully determined and the organisms whose permeability is to be tested be allowed to grow in it for a time, decrease in concentration of the medium may be interpreted to mean that absorption has taken place. This has been shown to occur in the case of a number of inorganic salts, but is especially well marked with solutions of glucose and glycerin. Demoussy[2] determined in this way the relative rate of absorption of potassium and calcium ions by wheat, maize, etc., while Laurent[3] was able to prove the somewhat unexpected fact, that roots of maize can absorb measurable quantities of glucose from a solution in which they are grown. This is probably not a general phenomenon, for if the protoplasm is permeable to sugar in one direction it is difficult to see how it could fail to be permeable in the opposite one also, and such a condition must allow outward diffusion of glucoses and other sugars which are found so commonly in plant cells.[4]

[1] O. H. Von Mayenburg, "Lösungsconcentration und Turgorregulation bei den Schimmelpilzen," *Jahrb. f. wiss. Bot.*, Vol. XXXVI (1901), pp. 381–420.

[2] E. Demoussy, "Absorption élective de quelques éléments minéraux par les plantes," *Compt. rend.*, Vol. CXXVII (1900), pp. 970–73.

[3] J. Laurent, "Sur l'absorption des matières organiques par les racines," *ibid.*, Vol. CXXV (1897), pp. 887–9.

[4] *Cf.* Pfeffer-Ewart, *Physiology of Plants*, Cambridge, 1900, p. 99.

That ordinary leaves can absorb inorganic salts was shown in this way by Dandeno.[1] He found that drops of solution placed upon foliage leaves were completely absorbed if too rapid evaporation was prevented. When the drop disappeared no trace of solute crystals remained upon the leaf surface.

d) Test by toxicity.— To all protoplasmic poisons must be accredited power of penetration in a greater or less degree; if there were no penetration the substance could not bring about its toxic effect. True[2] proved a slight toxicity for KNO_3 and NaCl upon Spirogyra; these substances must therefore penetrate the protoplasts of this plant, though probably this occurs with difficulty. More recently Coupin[3] has prepared a catalogue of the poisonous effects upon wheat of certain salts in various concentrations. His tables are useful for comparison. Of course, the fact that a rather high concentration of a given solute is needed to affect the plant may mean either that the protoplasm is only slightly permeable, or that the substance is only slightly toxic. From Pfeffer[4] we have the fact that mercuric chlorid and iodin penetrate many vegetable cells and exert a marked toxic effect.

There are many other proofs that various mineral and organic substances are able to penetrate the plant protoplast. Where a noticeable and specific effect is produced upon the organism by the presence of a given substance in the medium, there can be no doubt that the substance pene-

[1] J. B. DANDENO, "An Investigation into the Effects of Water and Aqueous Solutions of Some of the Common Inorganic Substances on Foliage Leaves," *Trans. Canad. Inst.*, Vol. VII (1901), pp. 238–350.

[2] R. H. TRUE, "The Physiological Action of Certain Plasmolyzing Agents," *Bot. Gaz.*, Vol. XXXVI (1898), pp. 407–16.

[3] H. COUPIN, "Sur la toxicité des composés du sodium, du potassium, et de l'ammonium à l'égard des végétaux supérieurs," *Rev. gen. bot.*, Vol. XII (1901), pp. 177–94.

[4] W. PFEFFER, *Osmotische Untersuchungen*, Leipzig, 1877, p. 140.

trates, to some extent at least. Whether the effect be the death of the plant or only an alteration in its metabolic processes makes no difference for the present consideration.[1]

e) *Test by accumulation.*—The power of penetration of all inorganic salts, and of many organic compounds also, may be tested by analysis of plant material which has been grown in the solution to be tested. The various metallic ions such as K, Na, Ca, etc., are known to accumulate in the bodies of higher plants. In this manner Bourget[2] demonstrated a marked absorption of iodin by various plant roots. There is a great difference in different plants in this regard, however; the Liliaceae and Chenopodiaceae absorb comparatively large quantities of iodin, while Solanum tuberosum, grown in the same soil, fails to absorb enough for a test.

Great accumulation of copper in plant cells has been recorded several times. Thus, MacDougal[3] describes a case where a tree of Quercus macrocarpa absorbed copper in large amounts and caused its precipitation within the wood in the metallic state.

All these accumulations come about by a chemical change taking place in the substance after it has entered the cell,

[1] The following references will be of use to supplement those already given: F. DE F. HEALD, "On the Toxic Effect of Dilute Solutions of Acids and Salts upon Plants," *Bot. Gaz.*, Vol. XXII (1896), pp. 125–53; H. M. RICHARDS, "Die Beeinflussung des Wachsthums einiger Pilze durch chemische Reize," *Jahrb. f. wiss. Bot.*, Vol. XXX (1897), pp. 665–79; F. L. STEVENS, "The Effect of Aqueous Solutions upon the Germination of Fungus Spores," *Bot. Gaz.*, Vol. XXVI (1898), pp. 377–406; E. B. COPELAND AND L. KAHLENBERG, "The Influence of the Presence of Pure Metals upon Plants," *Trans. Wisconsin Acad. Sci. Arts and Let.*, Vol. XII (1899), pp. 454–74; N. ONO, "Ueber die Wachsthumsbeschleunigung einiger Algen und Pilze durch chemische Reize," *Jour. Coll. Sci. Imp. Univ. Tōkyō*, Vol. XIII (1900), reviewed in *Bot. Gaz.*, Vol. XXX (1900), p. 422; H. DE VAUX, "De l'absorption des poisons métalliques très dilués par les cellules végétales," *Compt. rend.*, Vol. CXXXII (1901), pp. 717–20.

[2] P. BOURGET, "Sur l'absorption de l'iode par les végéteaux," *Compt. rend.*, Vol. CXXIX (1899), pp. 768–70; IDEM, same title, *Bull. soc. chim. Paris*, Ser. 3, Vol. XXIII (1899), pp. 40–41.

[3] D. T. MACDOUGAL, "Copper in Plants," *Bot. Gaz.*, Vol. XXVII (1899), p. 68. He cites the following on the same general subject: LEHMAN, "Der Kupfergehalt von Pflanzen und Thieren in kupferreichen Gegenden," *Arch. f. Hygien*, Vol. XXVII (1896), p. 1; J. B. SKEETSCHLY, "Tin Mines of Watsonville," *Report Geologist Queensland*, 1897.

either within the protoplasm or in the vacuole. If this were not so, the diffusion tension of the solute would soon become as great within the cell as without, and thus there could be no accumulation. But if a substance is precipitated, polymerized, or condensed within the cell through the chemical action of some other body already there, which perhaps arises as a secretion from the protoplasm, then the internal diffusion tension of the entering substance will be kept low, and inward diffusion will continue indefinitely. In this way copper salts entering the cell are probably reduced to metallic copper. This fact of accumulation is a very important one in understanding the process of absorption of dissolved substances by the plant.

f) Test by metabolic processes.—The absorption of any food substance is of course a proof of permeability to that substance. The immediate effect upon the living green cell of absence of carbon dioxid, or upon any living cell of oxygen, shows that these gases, when in solution, enter the protoplast with extreme ease. Penetration by many usually solid substances may be proved in this manner; the long series of experiments upon growth, and especially the formation of starch by green plants in darkness, may be regarded as evidence in this matter. Thus, Bouilhac[1] grew Nostoc in the dark in a solution of glucose, where it appeared perfectly healthy, and Artari[2] and Matruchot and Molliard[3] grew Stichococcus in organic solutions in a similar way. The absorption of organic food by many algæ, and by all saprophytes and parasites, including all of the fungi, may be mentioned in this connection. In many of these cases the

[1] R. BOUILHAC, "Sur la culture de Nostoc punctiforme en présence de glucose," *Compt. rend.*, Vol. CXXV (1897), p. 880.

[2] A. ARTARI, "Zur Ernährungsphysiologie der grünen Algen," *Ber. d. deutsch. bot. Ges.*, Vol. XIX (1901), pp. 7-10.

[3] L. MATRUCHOT ET M. MOLLIARD, "Variations de structure d'une algue verte sous l'influence du milieu nutritif," *Rev. gen. bot.*, Vol. XL (1902), pp. 114-30, 254-68, 316-32.

presence of the substance in question is due to digestion outside the body, brought about by outward diffusion of enzymes.

Dandeno[1] has recently shown that inorganic salts are absorbed in some instances by ordinary leaves when these are kept covered by a solution by means of a constant spray, or by submersion. This occurred to such a degree in this writer's experiments with Thunbergia that plants of this form whose roots were supplied with nothing but water, but whose leaves were sprayed with a solution, were able to make a good growth. Control plants, which had distilled water applied to the leaves as well as to the roots, perished in a much shorter time. Also, drops of solution placed upon various leaves were completely absorbed if too rapid evaporation was prevented. This observation has been mentioned under *c*).

g) *Outward permeability.*—Many substances which penetrate the cell from without have been shown to pass in the opposite direction with equal ease. This has been especially emphasized by Overton[2] in the case of the soluble alcohols, etc. But in general the outward passage from the plant body of sugars and the various organic food substances has not been demonstrated. It must be of rather rare occurrence, or the phenomena of nutrition, etc., would be impossible. There are, however, certain cases where exudation occurs, notably in the case of glandular structures, both in plants and animals. Laurent,[3] however, has demonstrated an outward passage of enzymes (*e. g.*, amylase and sucrase) from

[1] J. B. DANDENO, "An Investigation into the Effects of Water and Aqueous Solutions of Some of the Common Inorganic Substances on Foliage Leaves," *Trans. Canad. Inst.*, Vol. VII (1901), pp. 238–350.

[2] E. OVERTON, "Ueber die osmotischen Eigenschaften der lebenden Pflanzen und Thierzelle," *Vierteljahrschr. der Naturf.-Ges. in Zürich*, Vol. XL (1895), pp. 159–84.

[3] J. LAURENT, "Sur l'exosmose de diastases par les plantules," *Compt. rend.*, Vol. CXXXI (1900), pp. 848–51.

the roots of maize seedlings, and a similar phenomenon is very commonly met with in the case of bacteria, yeast fungi, etc. Molisch[1] believed this to be generally true, but Czapek[2] has shown that he was probably mistaken. The latter found that normal roots give off not only CO_2 but also phosphoric acid in the form of an acid salt. These substances must of course pass out through the protoplasm. There seems to be no doubt from the work of Dandeno[3] that both organic and inorganic substances will diffuse out from the cells of foliage leaves if these are kept covered with water. In these cases inward and outward diffusion seem to take place in exactly the same manner.

It is a well-known fact that enzymes, especially diastase, pass out from the cells of embryos and digest food stored in the endosperm of seeds.[4] This argues the permeability of the protoplasm of both embryo and endosperm to these substances. In this connection evidence has also been presented that the cells of embryo and endosperm are both permeable to carbohydrates, probably of the glucose group. Whether these arise from the action of an enzyme derived from the embryo, or from the action of enzymes formed within the endosperm itself,[5] is of no consequence so far as permeability is concerned; after the carbohydrates are formed they diffuse into the embryo.

h) *Variations in permeability.*—If a turgid cell gives

[1] H. MOLISCH, "Ueber Wurzelausscheidungen und deren Einwirkung auf organische Substanzen," *Sitzungsber. d. kais. Akad. d. Wiss. zu Wien*, math.-nat. hist. Klasse, Vol. XCVI (1887), pp. 84–109.

[2] F. CZAPEK, "Zur Lehre von den Wurzelausscheidungen," *Jahrb. f. wiss. Bot.*, Vol. XXIX, pp. 321–90.

[3] *Loc. cit.*, p. 71.

[4] GRUSS, "Ueber d. Eintritt von Diastase in d. Endosperm.," *Ber. d. deutsch. bot. Ges. zu Berlin*, Vol. IV (1893), p. 286; also B. HANSTEEN, "Ueber die Ursachen der Entleerung der Reservestoffe aus Samen," *Flora*, Vol. LXXIX (1894), pp. 419–29.

[5] K. PURIEWITCH, "Ueber die selbstthätige Entleerung der Reservestoffbehälter," *Ber. d. deutsch. bot. Ges.*, Vol. XIV (1896), pp. 207–15; IDEM, "Physiologische Untersuchungen über die Entleerung der Reservestoffbehälter," *Jahrb. f. wiss. Bot.*, Vol. XXXI (1897), pp. 1–76.

out *pure water*, this occurrence must be due to one of two conditions: (1) the protoplasm may contract with great force, thus overcoming the osmotic pressure of the contained solutes and causing the solvent to pass outward through the membrane, or (2) a relative decrease in the internal pressure may occur resulting either from an active precipitation or condensation of some of the solutes of the sap, or from an absolute rise in the external osmotic pressure. According to supposition (1), the osmotic pressure within the cell remains unchanged, but is in part overcome by the mechanical pressure of the contracting protoplasmic membrane. According to supposition (2), the internal osmotic pressure is relatively reduced, and the protoplasm does not exert any appreciable pressure itself, but is forced inward through the solvent by the osmotic pressure of the solutes outside the cell and by the elastic force of the restraining cellulose wall. It is probable that this last supposition expresses the truth in many cases where an alteration in turgidity is observed. The former supposition is not tenable at all; the protoplast would burst long before concentration of the sap solution could be brought about by pressure.

If a cell gives out a *solution*, the cause of this must be a change in the permeability of the protoplasm, such that it now allows the outward passage of solutes to which it was formerly impermeable. The liquid exuded in guttation is known[1] to be, not pure water, but a portion of the cell sap. In the last particular this sort of shrinkage of the vacuole differs from true plasmolysis, for in that we have the extraction of pure water. However, the apparent effect upon the cell is the same; if the volume of the vacuole is in any way decreased, the protoplasmic sac will contract from its own elasticity and surface tension, if for no other reason.

[1] G. BONNIER, "Recherches expérimentales sur la miellée," *Rev. gen. bot.*, Vol. VIII (1896), pp. 1-22; DANDENO has shown that guttation droplets contain both organic and inorganic solutes. See *Trans. Canad. Inst.*, Vol. VII (1901), pp. 238-350.

At different times and under different conditions the permeability of certain protoplasts apparently changes greatly. The presence of poisons may cause the protoplasm to become more permeable to other substances. Thus, Maquenne[1] found that $HgCl_2$ caused a marked increase in the permeability of the protoplasm of the cells of Helianthus seedlings to plasmolyzing agents. Similarly DeVries[2] found that plasmatic membranes which were normally impermeable to KNO_3 and NaCl could often be made permeable to them by treatment with an acid or a base. With animal muscle Loeb[3] has shown that acids, bases, and other chemicals exert a great influence upon the water-absorbing power of the cells. This may be due to changes in permeability.

Partial or complete plasmolysis may act in the same way. Oltmanns[4] was able to cause Fucus cells to give out coloring matter by placing the tissues in concentrated solutions. Both of these reactions must consist in an alteration of the physical (perhaps chemical) structure of the protoplasm. On the other hand, an increase in turgor above the normal may cause the same change. This seems to be the case in the guttation from the water pores of the leaves of the tomato, balsam, etc. When the turgor pressure in the cells bordering these water pores passes a certain limit, the protoplasm apparently becomes altered so that the cell sap oozes out and appears in droplets on the leaf-tips where water pores are present. Czapek[5] describes a similar phe-

[1] L. MAQUENNE, "Sur la pression osmotique dans les graines germées," *Compt. rend.*, Vol. CXXIII (1896), pp. 898, 899.

[2] H. DE VRIES, "Plasmolytische Studien ü. d. Wand d. Vacuolen," *Jahrb. f. wiss. Bot.*, Vol. XVI (1885), pp. 465–598.

[3] J. LOEB, "Physiologische Untersuchungen über Ionenwirkung": I. Mittheilung, "Versuche am Muskel," *Pflügers Arch. f. d. ges. Physiol.*, Vol. LXIX (1897), pp. 1–27.

[4] F. OLTMANNS, "Ueber die Bedeutung der Concentrationsänderung des Meerwassers für das Leben der Algen," *Sitzungsber. d. k. preuss. Akad. d. Wiss. zu Berlin*, Vol. X (1891), p. 183.

[5] F. CZAPEK, "Zur Lehre von den Wurzelausscheidungen," *Jahrb. f. wiss. Bot.*, Vol. XXIX (1896), pp. 321–90.

nomenon in the case of turgid root hairs, from which droplets of solution are exuded. It is probable that in these cases we have to do with a phenomenon related to glandular secretion.

Another potent cause for great increase in protoplasmic permeability in some instances is lowering of temperature. If a filament of any common alga be carefully dried externally and placed in olive oil whose temperature is then rapidly lowered to the vicinity of 0° C., a film of water may be seen to form about the filament, and partial plasmolysis may be observed. When the temperature is again brought back to normal, the extruded water is again absorbed. Greeley[1] has recently shown, not only that complete plasmolysis can be produced in Spirogyra by low temperature, but that the same thing occurs in Stentor coeruleus. Exactly the same phenomenon is exhibited by Stentor individuals when water is removed from them by the action of a concentrated sugar solution. The animals plasmolyzed by low temperature return to their normal activity with rise in temperature, but Greeley was unable to cause the same reversal in the case of the osmotically plasmolyzed individuals. I have often observed that the liquid exuded from cells of Spirogyra plasmolyzed by cold is a solution. Its freezing point is considerably lower than that of pure water.

The theory of death by freezing which was advanced by Molisch[2] accounts for the decline of activity and for final death at low temperatures by the extraction of water from the protoplasm until the processes which make up life are no longer possible. Matruchot and Molliard[3] have pointed

[1] A. W. GREELEY, "On the Analogy between the Effects of Loss of Water and Lowering of Temperature," *Am. Jour. Physiol.*, Vol. VI (1901), pp. 112–28.

[2] H. MOLISCH, *Untersuchungen über das Erfrieren der Pflanzen*, Jena, 1897; reviewed in *Bot. Centralbl.*, Vol. LXXIII (1898), p. 149.

[3] L. MATROCHOT AND M. MOLLIARD, "Sur l'identité des modifications de structure produites dans les cellules végétales par le gel, la plasmolyse, et la fanaison," *Compt. rend.*, Vol. CXXXII (1901), pp. 495–8.

out a striking parallelism in the behavior of plant nuclei which have been either frozen, dried, or subjected to the osmotic action of a concentrated solution. In all these cases water was found to be extruded from the nucleus. The nuclear material took on a peculiar appearance not unlike that of karyokinetic figures.

Krabbe[1] experimented upon the effect of rise in temperature upon the absorption of water by various plant cells, finding that the rate of absorption rises with the temperature. This author supposes the response to be due to a physical change in the protoplasm, caused by the higher temperature.

But the best series of experiments on the change in protoplasmic permeability due to temperature variations is that of van Rysselberghe.[2] He worked with a variety of plant cells (Sambucus, Tradescantia, Begonia, Lemna, green algæ, etc.) and found that Krabbe's general result is true. The ratio of increase in permeability to water becomes less, however, as the temperature rises. From 0° C. to 5° C., this ratio is 0.05; from 5° C. to 18° C., 0.043; and above the last-named temperature, 0.1. The ratios between the permeability to water at

0° and that at 6°, 12°, 16°, 20°, 25°, 30°

are: 1, ... 2, 4.5, 6, 7, 7.5, 8.

The total amount of water absorbed by a cell is not changed by variations in temperature; the rate of absorption alone is affected. The nature of the protoplasm does not appear to have any effect on the total amount of water absorbed or given out by a cell; this is determined by the osmotic pressure of the sap and by the temperature. Permeability to

[1] G. KRABBE, "Ueber d. Einfluss d. Temperatur auf d. osmotische Processe lebender Zellen," *Jahrb. f. wiss. Bot.*, Vol. XXIX (1896), pp. 441–98.

[2] F. VAN RYSSELBERGHE, "Influence de la température sur la perméabilité du protoplasme vivant pour l'eau et les substances dissoutes," *Recueil de l'inst. bot. de Bruxelles*, Vol. V (1901), pp. 209–49; IDEM, "Réaction osmotique des cellules végétales à la concentration du milieu," *Mem. cour. pub. par l'acad. roy. de Belg.*, Vol. LVIII (1898), pp. 1–101.

this liquid does not cease altogether at 0° C., as was thought by Schwendener.[1] At 0° C. protoplasm is not only permeable to water, but also to KNO_3, glycerin, urea, methylene blue, caffein, and ammonium carbonate. Thus Krabbe's idea that below 5° C. nothing but water penetrates is entirely unfounded. Temperature variations in permeability to solutes were also observed in many cells by the same author. These, too, seem to follow the rule for water as stated on the preceding page.

However, Copeland[2] showed that decrease in temperature caused a rise in tde turgor pressure of moss leaves. This may not be a direct effect of the temperature upon the protoplast, for the same author found that various agencies which checked growth also caused a rise in turgor; there is surely a close relation between growth and turgor. whatever this relation may ultimately turn out to be. In this connection it may be noted that De Vries[3] found that, as growth proceeds, turgor rises, to fall again after the curve of growth begins to decline.

The extrusion of liquid from the cells of the pulvini of "sensitive" organs, such as the leaves of Mimosa and the stamens of Berberis, may be due to a change in permeability also. There seems to be a question, however, as to whether the exuded liquid is water or a solution. Pfeffer[4] considers this subject at some length, and concludes that solutes probably do not pass out. He believes that the salts found by Janse[5] in the extruded liquid from pulvini in Mimosa are

[1] S. SCHWENDENER, "Zur Kritik der neuesten Untersuchungen ü. d. Saftsteigen," *Sitzungsber. d. k. preuss. Akad. d. Wiss. zu Berlin*, Vol. VI (1892), p. 911.

[2] E. B. COPELAND, *Ueber den Einfluss von Licht u. Température auf den Turgor*, Halle a. S., 1896.

[3] H. DE VRIES, "Ueber die Ausdehnung wachsender Pflanzenzellen durch ihren Turgor," *Bot. Zeitg.*, Vol. XXXV (1877), p. 1.

[4] W. PFEFFER, "Zur Kenntniss der Plasmahaut u. d. Vacuolen, etc.," *Abhandl. d. k. sächs. Ges. d. Wiss.*, math.-physik. Klasse, Vol. CLXI (1890), pp. 187–344.

[5] J. M. JANSE, "Die Permeabilität des Protoplasma," *Verslag. en Mededeel. d. k. Akad. v. Wetensch. te Amsterdam*, 1888.

merely extra-cellular material. But Pfeffer shows that there is surely an extrusion of solutes from the stamens of Cynara. It may be that all "sensitive" organs do not act alike in this regard. Hilburg[1] states that the cells of the pulvini of leaves of Phaseolus increase in turgor pressure when subjected to the action of light.

Puriewitch[2] found that absence of oxygen and the presence of anæsthetics prevented the giving off of reserve food from cells of tubers, roots, bulbs, the endosperm of seeds, etc. He explains this fact as an effect upon the diastatic enzymes in the various cases, but the onion bulb and the beet root exhibit the same phenomenon, and it is difficult to see how organs whose stored food is already in solution could be affected by an alteration in enzymes—unless, indeed, even sugars need to be modified by enzyme action before they can pass the protoplasm. A live onion scale totally submerged in water will give off no sugar, but if partially exposed to the air exudation takes place. It is probable that here we have another case of the influence of a chemical (*i. e.*, oxygen) upon the permeability of the membrane. That enzyme action is necessary for the translocation of the cane sugar of the beet root is possible and even probable.

The permeability of the protoplast to a certain substance may change according to the relative concentration of that substance within and without the cell, as has been shown recently by Nathansohn.[3] Codium tomentosum was used in his experiments. The cells of this plant are permeable to chlorids, so that the content of HCl is found to be the same in expressed sap and in the surrounding medium. If the

[1] C. HILBURG, "Ueber Turgescenzänderungen in den Zellen der Bewegungsgelenke," *Unters. aus d. bot. Inst. zu Tübingen*, Vol. I (1881), pp. 23–52.

[2] K. PURIEWITCH, "Physiologische Untersuchungen ü. d. Entleerung der Reservestoffbehälter," *Jahrb. f. wiss. Bot.*, Vol. XXXI (1897), pp. 1–76.

[3] A. NATHANSOHN, "Zur Lehre von Stoffaustausch," *Ber. d. deutsch. bot. Ges.*, Vol. XIX (1901), pp. 509–12.

plant be put into a solution without the chlorid, this substance diffuses out of the cells in twelve to twenty-four hours, and the reverse occurs if the external solution contains more Cl ions than the internal. Other substances in the medium will cause the retention of chlorid, apparently in inverse proportion to their ability to penetrate. The following table to show this is taken from Nathansohn's paper:

Medium	Pressure	HCl Content per cent.
Normal Sea H_2O	Sw[1]	2.25
Na NO_3	$\frac{1}{2}$Sw	.67
Na NO_3	Sw	1.27
Urea	Sw	0.64
Glycerin	Sw	1.0
Grape sugar	$\frac{4}{5}$Sw	1.45

Of the substances named in the table, those of least penetrating power are grape sugar and $NaNO_3$. This is a new departure, and no definite conclusions can be drawn until more work has been done.

Very clear evidence of change in permeability of protoplasm to solutes is that obtained by Haupt[2] in his work on extrafloral nectaries. In certain plants (*e. g.*, Euphorbia and Vicia) he found that the secretion of sugar by the nectaries was profoundly influenced by light, specifically by the red-yellow rays of the sun's spectrum. Secretion of sugar occurred only in light, and from nectaries already containing sugar this substance was resorbed in darkness or in blue light. This must indicate that the nature of the protoplasmic membranes here is entirely altered by the etheric vibrations; in light sugar passes outward, while in darkness it moves in the opposite direction. That plants which had

[1] Sw in the table denotes the osmotic pressure of normal sea-water.

[2] H. HAUPT, "Zur Secretionsmechanik der extrafloralen Nektarien," *Flora*, Vol. X (1902), pp. 1–41.

been deprived of CO_2 for many days still exhibited this response seems to show that the phenomenon stands in no direct relation to the process of photosynthesis.

IV. ACTION OF THE PROTOPLASMIC MEMBRANE

If ignorance still prevails regarding the manner in which a purely physical osmotic membrane acts, it is even more prevalent regarding the action of the protoplasmic membrane of the living cell. That the tonoplast and ectoplast are the main factors in the production of semi-permeability there seems little reason to doubt. There are three possible ways in which they may act, and of course the same membrane may act in different ways at the same time. These three possible explanations of semi-permeability may be briefly stated as follows:

a) *The filter theory.*—The simplest explanation of osmotic pressure, whether within a living cell or not, is this: That the semi-permeable membrane acts merely as a sieve or filter, and that the larger solute particles are prevented from passing by the smallness of the opening, while the smaller solvent particles pass with only slightly increased friction. Since we cannot be sure of the relative sizes of the different molecules and ions, there is no way of directly testing this hypothesis. But there are many facts, both in chemistry and physiology, which make the filter theory at least very improbable in many instances. Among the physiological facts which tend in this direction may be cited the effects of chemicals in varying the permeability of the protoplasm, especially the observations of Maquenne, De Vries, and Loeb, already discussed[1] and the work of Overton, to be taken up under *b*).

b) *The solution theory.*—According to this theory, the membrane is to be considered as a solvent in which the pene-

[1] See above, p. 74.

trating substances are readily soluble, while those which fail to penetrate are not. Thus, in the case of an osmotic cell containing a sugar solution and surrounded by pure water, it may be supposed that the membrane dissolves water readily, but cannot dissolve sugar. Thus, water would go into solution in the membrane on the side of higher diffusion tension of water (the side of the solvent), and, after diffusing through the membrane, would be given off on the side of lower diffusion tension of that substance (the side of the solution). The solute, not being able to go into solution in the membrane, merely exerts its expansive force upon it, and does not diffuse.

From the researches of Overton[1] and others it appears that this is probably the true explanation of many cases of the development of osmotic pressure in plant and animal cells. By comparing the numerous substances which he found able to penetrate the protoplasm, Overton observed that penetrating power seemed to vary in proportion to solubility in aliphatic oils and ethers. He went still further: A given substance, like glycerin, may be only slightly soluble in aliphatic oils, and may penetrate the protoplast slowly; but if its substitution products (as mono- and dichlor-hydrin, or mono- or dimethyl-glycerin) are more soluble in this class of substances, their power of penetrating the plant protoplast is also found to be greater, and this in proportion as solubility has been increased by the substitution. From these and many other observations Overton is led to conclude that the peculiar property of the protoplast of being permeable to certain substances and not to others probably depends upon the presence within it of some aliphatic oil or ester, or a mixture of these substances. He goes so far as to point out that cholesterin and the lecithins, substances

[1] E. OVERTON, "Ueber die allgemeinen osmotischen Eigenschaften der Zelle, ihre vermuthlichen Ursachen und ihre Bedeutung für die Physiologie," *Vierteljahrschr. d. Naturf.-Ges. in Zürich*, Vol. XLIV (1899), pp. 88–135.

of this nature which are of general occurrence in animal and plant cells, when in certain mixtures, absorb water. He suggests that the tonoplast and ectoplast may be merely layers of such substances, and gives reasons why it is not plausible to suppose that a simple aliphatic oil plays this part of surface layer, as was supposed by Quincke.[1] The difference, however, between oils and lecithins is so slight that Quincke's theory is not fundamentally modified by these facts.

On the purely physical side Meerburg's work[2] on the passage of fuchsin through membranes of copper ferrocyanid seems to present evidence in favor of the solution theory. The dye could not pass the membrane, even in a slight degree, until the latter had become fully impregnated with it. Flusin also showed[3] that the rapidity of diffusion of various substances through a membrane of caoutchouc varies in the same degree as does the absorptive power of the membrane for these substances. The same was found to be true for pig's bladder.

c) *The chemical theory.*—The chemical explanation of the phenomenon of semi-permeability supposes that the membrane takes an active chemical part in the transmission of substances from one side to the other. In all its freedom of indefiniteness this theory offers the only escape from the dark problem of glandular secretion, wherein the solute moves from the weaker to the stronger solution against its own diffusion tension. This is one of the most difficult of physiological problems, and upon it absolutely no light has

[1] QUINCKE, "Ueber Emulsionsbildung und d. Einfluss der Galle bei der Verdauung," *Pflügers Arch. f. d. ges. Physiol.*, Vol. XIX (1879), pp. 129–44.

[2] J. H. MEERBURG, "Zur Abhandlung Tammanns: Ueber die Permeabilität der Niederschlagsmembranen," *Zeitschr. f. physik. Chem.*, Vol. XI (1893), p. 446–8.

[3] G. FLUSIN, "Sur l'osmose des liquides à travers une membrane de caoutchouc vulcanisé," *Compt. rend.*, Vol. CXXVI (1898), pp. 1497–1500; IDEM, "Sur l'osmose des liquides à travers une membrane de vessie de porc," *ibid.*, Vol. CXXXI (1900), pp. 1308, 1309.

yet been shed. In the realm of pure physical chemistry there is some evidence that osmotic membranes may sometimes play a chemical part.[1]

V. THE NATURE OF THE OSMOTICALLY ACTIVE SOLUTES

Within the cell sap there are very many substances in solution, and thus the question as to what substances the turgor pressure is due becomes of some importance. DeVries[2] found that in the onion bulb-scale and in the beet root this pressure is chiefly due to stored sugar. He also showed that the salts KCl and KNO_3 play important rôles in the maintenance of turgor in leaf-stalks of Gunnera scabra and the shoots of Helianthus tuberosus, respectively. Copeland[3] concluded from experiments with Phaseolus, Pisum, Fagopyrum, and Zea, that in these plants the osmotically active substance is mainly potassium nitrate. On the other hand, Kraus[4] and DeVries[5] found that in many plants organic acids are the main source of the osmotic pressure of the cell sap. The research of von Mayenburg[6] shows that Aspergillus

[1] On the action of animal membranes in diffusion phenomena see the following: H. J. HAMBURGER, "Die isotonischen Koeffizienten und die rothen Blutkörperchen," *Zeitschr. f. physik. Chem.*, Vol. VI (1890), pp. 319–33; IDEM, "Ueber den Einfluss des intra-abdominalen Druckes auf die Resorption in der Bauchhöhle," *Arch. f. Anat. u. Physiol.*, Physiol. Abth., Jahrg. 1896, p. 302, *ibid.*, p. 428; G. KÖVESI, "Beiträge zur Lehre der Resorption im Dünndarm," *Physiol. Centralbl.*, Vol. XI (1898), pp. 555–7 and 593–7; O. COHNHEIM, "Ueber Dünndarmresorption," *Zeitschr. f. Biol.*, Vol. XXXVI (1898), pp. 129–53; S. G. HEDIN, "Ueber den Einfluss einer thierischen Membran auf die Diffusion verschiedener Körper," *Pflügers Arch. f. d. ges. Physiol.*, Vol. LXXVIII (1899), pp. 205–61; R. HÖBER, "Ueber Resorption im Dünndarm," *ibid.*, Vol. LXXIV (1899), pp. 246–71. On the action of the copper ferrocyanid membrane see G. FLUSIN, "Sur l'osmose à travers la membrane de ferrocyanure de cuivre," *Compt. rend.*, Vol. CXXXII (1901), pp. 1110–12.

[2] H. DE VRIES, "Eine Methode zur Analyze der Turgorkraft," *Jahrb. f. wiss. Bot.*, Vol. XIV (1884), pp. 427–60.

[3] E. B. COPELAND, "The Relation of Nutrient Salts to Turgor," *Bot. Gaz.*, Vol. XXIV (1897), pp. 399–416.

[4] G. KRAUS, *Stoffwechsel der Crassulaceen*, 1886.

[5] H. DE VRIES, "Ueber d. Bedeutung d. Pflanzensäuren," etc., *Bot. Zeitg.*, Vol. XXXVII (1879), pp. 847–53; IDEM, "Ueber d. Antheil Pflanzensäuren," etc., *Bot. Zeitg.*, Vol. XLI (1883), pp. 849–54.

[6] O. H. VON MAYENBURG, "Lösungsconcentration und Turgorregulation bei den Schimmelpilzen," *Jahrb. f. wiss. Bot.*, Vol. XXXVI (1901), pp. 381–420.

niger, when grown in strong nutrient solutions of sugar, etc., escapes plasmolysis by an enormous increase in concentration of the cell sap, this being produced in part by true carbohydrates, but mainly by some unidentified substance, which is, however, probably closely related to the latter group. Maquenne[1] found by the freezing-point method that the expressed juice of seedling peas six days old had an osmotic pressure such that the average molecular weight of the solutes must be in the neighborhood of 239. This shows that the active substance has a much larger molecule than glucose (mol. wt. 180). The juice failed to show the presence of glucose or cane sugar by tests made with Fehling's solution and with phenylhydrazin acetate. Helianthus seedlings, however, showed the presence of glucose, and the sap had an average molecular weight of only 136. Here, then, the pressure is largely produced by some molecule much smaller than that of glucose. Stange[2] found that the cells of growing root tips of Pisum, Lupinus, etc., which had been grown in strong KNO_3 solutions, showed no accumulation of that salt, although there was a marked increase of it in the parenchyma farther up. The turgor pressure was the same in the root tip as elsewhere in the plant. This argues that the increase in turgor in the growing region, which prevents plasmolysis where the culture is in a strong solution, must be due to some other substance or substances. A collection of analyses of various plant parts, which may be taken as at least some indication of the nature of the active substances in the sap, is given by Mayer.[3] DeVries[4] made

[1] L. MAQUENNE, "Sur la pression osmotique dans les graines germées," *Compt. rend.*, Vol. CXXIII (1896), pp. 898 ff.

[2] B. STANGE, "Beziehungen zwischen Substratconcentration, Turgor und Wachsthum bei einigen phanerogamen Pflanzen," *Bot. Zeitg.*, Vol. L (1892), p. 253.

[3] A. MAYER, *Lehrbuch der Agriculturchemie*, Heidelberg, 1895, pp. 307 ff.

[4] H. DE VRIES, "Eine Methode zur Analyse der Turgorkraft," *Jahrb. f. wiss. Bot.*, Vol. XIV (1884), pp. 427–601.

analyses of the sap itself and his results show that different plants vary much as to the nature of their osmotically active substances.

Heald[1] has recently determined the electrical conductivity of sap expressed from the roots, stems, and leaves of various plants. The amount of electrolytes thus indicated is in reasonably close agreement with the amount of ash found by incineration. This only goes to show that most of the electrolyte molecules are dissociated in the sap, and are therefore active in conducting the current. Any conclusions with regard to the osmotic pressure of the sap which are based on conductivity methods must be absolutely unreliable, unless it is first ascertained that there are no non-conductors present, and also that the electrolytes present are in the ionic condition. But it is probably impossible to find a natural plant juice whose solutes are all electrolytes. Therefore Heald's method cannot be of use in determining osmotic pressures. The freezing-point, boiling-point, and vapor tension methods are applicable to this problem, however.

Maquenne's work on the freezing-points of plant juices[2] has recently been added to by Sutherst.[3] The latter author has merely given the freezing-points, without determining the weight per liter, so that his results will not be available for any determination of the nature of the solutes. In the following table, taken from his paper, I have calculated the osmotic pressures to facilitate comparison:

	Fr. Pt.	Os. Pressure in mm. of Hg.
Vegetable marrow:		
Leaf and stalk	−0.75°	6,880
Fruit	−0.75°	6,880
Swede turnip:		
Entire plant	−1.0°	9,173.2

[1] F. DE F. HEALD, "The Electrical Conductivity of Plant Juices," *Science*, N. S., Vol. XV (1902), p. 457; IDEM, same title, *Bot. Gaz.*, Vol. XXXIV (1902), pp. 81–92.

[2] See above, p. 84.

[3] W. T. SUTHERST, "The Freezing Point of Vegetable Saps and Juices," *Chem. News*, Vol. LXXXIV (1901), p. 234.

	Fr. Pt.	Os. Pressure in mm. of Hg.
Celery:		
Green stalk and leaf	−1.4°	12,842.48
White parts	−0.75°	6,880
Carrot:		
Leaf and stalk	−1.2°	11,007.84
Root	−1.0°	9,173.2
Cabbage:		
Outer leaf	−1.1°	10,090.52
Heart	−0.85°	7,797.22
Apple, fruit	−1.4°	12,842.48
Pear, fruit	−1.75°	16,053.2

From the evidence at hand it may therefore be concluded that there is great variability among different plants with regard to the particular substances called into requisition to maintain the turgor pressure. There seems to be a general tendency for these to be of an organic nature, and to possess rather complex molecules.[1]

VI. THE MAINTENANCE OF TURGIDITY IN SPITE OF PERMEABILITY TO CERTAIN SOLUTES

It might be supposed that the fact of greater or less permeability of the protoplasm to various solutes would lessen the value of the osmotic explanation of the phenomenon of turgidity. This, however, does not necessarily follow. While certain substances are diffusing in and out of a cell, its turgidity may be maintained by the presence within the vacuole of some other osmotic substance or substances to which the protoplast is impermeable, or very slightly permeable. It is probable that this is what occurs in living plant cells. These effective osmotic substances are usually of the nature of carbohydrates, plant acids, and mineral salts. They are probably secreted into the vacuole by the activity of the surrounding protoplasm. How this can occur is not yet known. The process must involve movement of

[1] *Cf.* PFEFFER-EWART, *Physiology of Plants*, Cambridge, 1900, p. 141.

solute particles from a lower concentration to a higher, against their own diffusion tension. It may be that these substances are freed from the protoplasm in a certain form, and that, after entering the vacuole, they polymerize or change their nature in some way, according to special conditions there existing. The accumulation of many substances within the vacuole (*e. g.*, anilin dyes[1] and caffein) is surely due to a chemical reaction after the substance has passed the protoplasmic layers.

VII. RELATION OF TURGIDITY TO VITAL ACTIVITY

Only because of the existence of the phenomenon of turgidity has the plant organism been able to develop into what it is. In several ways turgidity is absolutely essential, and in many others advantageous, to vital activity as it is now exhibited in plants.

a) *The retention of form.*—By means of turgor pressure the delicate fluid or semi-fluid plasma of the cell is kept pressed out against the cellulose wall, and the plasmic membranes are thus kept in a uniform state of tension, upon which condition some of their physical properties doubtless depend; as has been seen, for instance, great variations in turgor may so affect the protoplast as utterly to change its permeability to certain solutes.

b) *Mechanical support.*—All parenchymatous tissues and nearly all filamentous and uni-cellular forms owe most of whatever rigidity they possess to the stress set up between the internal osmotic pressure and the elastic force of the stretched cell walls. A heavy weight might be supported upon a pile of inflated footballs if they were properly placed, but if the individual balls were leaky they would no longer be available for such a purpose. In a similar manner the plasmolysis of any thin-walled tissue is accompanied by a

[1] *Ibid.*, pp. 119 ff.

more or less marked collapse. Also, the existence of differences in the turgor, and hence in the tissue tensions, of different parts of the body in higher plants, increases the mechanical strength of the whole structure to a very marked degree. The difference in tension between the pith and cortex of many herbaceous stems is an illustration of this fact.

c) *Growth.*—Exactly what the relation between growth and turgor may be cannot yet be stated, but there is good evidence to show that in the presence of turgidity growth is accelerated and in its absence retarded. Increase in thickness of the cell wall cannot take place unless the protoplast is kept turgid, and thus closely applied to it, as was shown by Reinhardt.[1] Other evidence along this line is that obtained by Klebs,[2] when he succeeded in causing a new cellulose wall to form within the old one by keeping the protoplast in a state of plasmolysis. Also the work of Bower[3] needs to be considered here. This author brings out very clearly the fact that there is a close connection between wall and protoplasm, by a study of the strands and fibers which remain joining the protoplast to the wall in a plasmolyzed cell. He thinks that the attachment of the protoplasm, which results in the formation of these strands, is closely connected with the process of wall-formation. The strands are not usually opposite on the two sides of a common wall, and thus apparently have no relation to pores through the wall.

Experiments on the effect of light and temperature led Copeland[4] to the conclusion that growth regulates turgor rather than turgor growth.

[1] M. O. REINHARDT, "Plasmolytische Studien zur Kenntniss des Wachsthums der Zellmembran," *Festschrift für Schwendener* (1899), p. 425.

[2] G. KLEBS, "Beiträge zur Physiologie der Pflanzenzelle," *Unters. aus d. bot. Inst. zu Tübingen*, Vol. II (1888), pp. 489–568.

[3] F. O. BOWER, "On Plasmolysis and its Bearing upon the Relations between Cell-wall and Protoplasm," *Quart. Jour. Microsc. Sci.*, Vol. XXIII (1885), pp. 157–67.

[4] E. B. COPELAND, *Ueber d. Einfluss von Licht u. Temperatur auf den Turgor*, Halle a. S., 1896.

d) *Curvature.*—Since most plant curvatures are due to modified growth, it is to be expected from what has just been said that turgor must play an important part in these phenomena. A discussion of the relations of this question will be found in Pollock's paper[1] on root curvature. To enter into this much mooted question would be going too far afield from the present subject.

Other more rapid curvatures (such as those of the pulvini of the leaves of the Mimosa, the stamens of Berberis, various tendrils, etc.), which are not due to growth, have already been mentioned. They owe their existence entirely to turgor changes.

e) *Work.*—Turgor is also of great benefit to the plant in that it gives it a means of doing work, of overcoming resistance. The lifting of sidewalks and buildings and the splitting of cliffs by the roots of trees are examples of the extent to which this pressure may be developed. For it must be remembered that the growing region of any plant is always mechanically weak; it owes practically all its power of resistance to the turgidity of the cells. Pfeffer[2] has made many tests and measurements in this field, and Rodewald[3] has applied mathematics to the problem, showing how osmotic pressure is a very considerable source of energy to the plant. Of course the energy ultimately comes from the heat of external objects.

VIII. SUMMARY OF THE CHAPTER

Turgidity is the immediate result of osmotic pressure within the cell. It arises from pressure developed within the cell sap of solutes which are unable to penetrate the surrounding

[1] J. B. POLLOCK, "The Mechanism of Root Curvature," *Bot. Gaz.*, Vol. XXIX (1900), pp. 1–63.

[2] W. PFEFFER, "Druck und Arbeitsleistung durch wachsende Pflanzen," *Abhandl. d. k. sächs. Ges. d. Wiss. zu Leipzig*, math.-physik. Klasse, Vol. XX (1893), p. 235.

[3] H. RODEWALD, "Ueber die durch osmotische Vorgänge mögliche Arbeitsleistung der Pflanzen," *Ber. d. deutsch. bot. Ges.*, Vol. X (1892), pp. 83–93.

plasmatic membranes or which penetrate them very slowly, the concentration (and hence the pressure) being greater within the cell than in the external solution. The internal concentration is probably kept up by the chemical activity of the protoplasm itself, substances of the nature of soluble carbohydrates or organic acids being formed and secreted into the vacuole. How such a movement of solutes against the direction of their own diffusion tension can occur is not yet explained. Perhaps they change their nature after leaving the protoplasmic layer and entering the vacuole. Turgor pressure may vary in different cells through wide limits (from less than two to more than a hundred atmospheres) and in the same cell the variation during different periods of growth may be almost as great. Turgidity is influenced by variations in the amount of water at hand and also by various conditions which affect the permeability of the protoplasm directly.

CHAPTER II

ABSORPTION AND TRANSMISSION OF WATER

I. ABSORPTION OF WATER

As has been seen in the previous chapter, it is absolutely essential that every living mass of protoplasm be saturated with water. This is so primarily on account of the fact that the energy transformations which are designated as vital phenomena occur solely in aqueous solutions. It is also true because of the fact that water is actually used in these transformations; it is chemically combined with other substances to form carbohydrates, proteids, etc. Therefore it becomes necessary that every active cell be not only saturated with water, but also that it be in connection with an external supply of this material. Especially is this so where water is lost by evaporation. It is possible, of course, for a plant cell to become hermetically sealed within a water-proof wall (*e. g.*, fungus spores, etc.), but as long as it is active and growing it cannot be so shut off from the outward supply of water.

If the cell be naked and immersed in water, the supply of this substance is always at hand and simply diffuses into the protoplasm as it is used in metabolism. If the organism be in contact with a moist substratum throughout most of its surface, as is the Myxomycete plasmodium, the absorption of water takes place from the imbibed water of the substratum. When the cells are surrounded by cellulose membranes, these are kept saturated by diffusion from without, and the protoplasm absorbs its needed water from them. The cellulose walls of ordinary cells act like the porous and imbibed substratum against which the Myxomy-

cete plasmodium lies. Thus, if the plant body is not extensive and is mostly in contact either with free aqueous solution or with some moist substance, the problem of how it obtains water is a simple one. Also, mere diffusion from one part of the organism to another, and that for only short distances, is sufficient to account for all the water transport to be met with in such plants.

But where the plant body elongates upward from its substratum (a phenomenon occurring in all forms, from the sporophores of fungi to the stems of higher plants), it becomes more difficult to point out exactly how every living cell is kept saturated with water. In the case of fungi the rhizoidal part of any filament is in direct contact with the substratum, and here the solution of the substratum is continuous with that of the protoplasm, through the saturated cellulose membrane. The sporophore is in connection with absorbing rhizoids and through these it absorbs not only what water is needed for growth, metabolism, etc., but also what is needed to replace that lost by evaporation. For if the loss by evaporation is not made up, death, or at least suspension of activity, must ultimately ensue from desiccation. The cellulose walls which are exposed to the air are more or less thickened, thus causing water loss by evaporation to be much less pronounced than it would otherwise be. Indeed, fungi are seldom found growing in localities where evaporation is very marked. In the case of algal filaments, which grow upward from the substratum, and in that of elevated capsules in liverworts and mosses, water transport is to be explained in the same way.

In the higher green plants, except in the case of aquatic plants, conditions become much more complex. Here the most active parts, the leaves and expanding buds, are often removed many meters from the soil out of which the supply of water must come. In these plants special organs of

absorption, the roots, and a special region of transmission of water, the xylem, have been developed.

But the great expanse of exposed surface would render it utterly impossible that the living cells of one of the larger plants be kept saturated with water, were it not for the development of various kinds of thickened walls and even layers of dead cells (*e. g.*, cork) covering the exposed surfaces. By these means evaporation is greatly reduced. But to carry on the photosynthetic process it is essential that carbon dioxid be freely absorbed from the air. Now the only manner by which this gas can reach the interior of the living cells is by passing into solution in the imbibed water of the cellulose walls and then diffusing through the tissues as a solute. The complicated structures of stomata and air chambers bring about a condition of things such that thoroughly saturated cellulose walls are exposed to the air, while at the same time a minimum amount of evaporation is allowed to go on. The plant would be a more economical machine, in some ways at least, if it could avoid evaporation entirely, but this is impossible if imbibed walls are to be exposed to the atmosphere. By far the greater quantity of the water absorbed through the roots finds its way out of the green plant through the leaves, and is of no direct material use. This evaporation plays an important part in keeping the green parts cool when they are subjected to the direct rays of the sun. It is probable also that much of the energy for raising water in the plant comes from this molecular diffusion, which we call evaporation. The same process produces a current of liquid up the stem and thus aids in the transmission of solutes.

A comparatively small amount of the absorbed water is used as food material in the processes of photosynthesis, growth, and general metabolism. In the lower forms without chlorophyll the exposure of a wet membrane to the atmos-

phere is not so essential, nor is it essential that an extensive surface be exposed at all. A comparatively small area will suffice for the evaporation of the gaseous products of respiration, etc., or these may be allowed to diffuse outward into the solutions of the substratum. Thus, in colorless parasites and saprophytes are found reduced surfaces covered with leathery tissues which contain few or no openings. However, water is so plentiful in some habitats that many of the forms found in such places have never acquired any special protection against evaporation.

In aquatics the effects of evaporation are not present; absorption and transmission of water take place by direct diffusion, perhaps mainly through the roots, however.

Since both cell wall and protoplasm are permeable to water, this substance will diffuse into a cell when, for any reason, its diffusion tension is less within than it is outside. Thus, if the surrounding medium is a very dilute solution, all cells must absorb water until an equilibrium is established between the expanding solutes within the vacuole and the elastic cellulose wall without. By imbibition the cell wall is kept saturated with water also, so that there is direct water communication between adjacent cells even where there is no protoplasmic connection.

This form of water absorption is universal in all organisms. It makes no difference how complex the form, the individual cells stand in the same relation to water external to them as does the Myxomycete plasmodium moving about in its moist substratum. Of course in the higher forms the cells are fixed in position. In the latter the important special condition is that here the moist substratum in which any cell lives is often the adjacent living tissue of the same plant. In a complex tissue, if one cell loses water faster than its neighbors, water diffuses from them into it and equilibrium is maintained.

In all organisms except the very lowest the power to absorb moisture from outside the body is possessed by only relatively few cells, whose external position fits them for this. Thus, some submerged aquatics may perhaps absorb equally throughout the whole extent of their comparatively thin epidermis. Partially submerged forms can absorb only through those surfaces which are under water. Ordinary land plants absorb through the surface layers of the younger portions of their roots, the surface layer being often greatly extended by the development of root hairs. But in any event, no matter where the water passes from the substratum into the plant body, absorption always takes place in the same way. The cellulose membranes are kept wet by imbibition, and water diffuses into the protoplasm from them. The forces which cause the entrance of water into the plant are, then, partly those of adhesion and surface tension, and partly those of simple diffusion.

That the rate of root absorption varies with the temperature of the soil when the changes in temperature are gradual, as was demonstrated by Vesque,[1] shows that this absorption is an osmotic phenomenon. With a sudden rise in temperature this author found that absorption is diminished temporarily, and with a sudden fall it is augmented. This is probably due to the increased pressure of the inclosed gas bubbles at higher temperatures.

II. TRANSMISSION OF WATER

a) *Water loss.*— Within the single cell transmission of water occurs mainly by simple diffusion, aided, no doubt, by streaming movements of the protoplasm. In more complex forms, like liverworts, and in simple tissue masses of the higher plants, diffusion from cell to cell through the sat-

[1] J. VESQUE, "De l'influence de la température du sol sur l'absorption de l'eau par les racines," *Ann. sci. nat. bot.*, Ser. VI, Vol. VI (1877), pp. 169–209.

urated walls brings about all the transfer that is required. The process may often be hastened by pits and protoplasmic connections. In higher plants phenomena occur which are more difficult of explanation. In order to discuss the transmission of water in such cases, it will first be necessary to consider briefly the ways in which water is lost by the plant.

(1) *Evaporation.*— As has been stated already, by far the greater part of the water absorbed by the plant is lost by evaporation. Water is continually evaporating into the air-spaces of the plant body and diffusing out into the external atmosphere through stomata and lenticels, and to some extent through the epidermis itself. The vacuoles of the leaf parenchyma furnish this water to the cell walls, and thus their solutions become more concentrated as evaporation continues. Of course this means that water must diffuse into these cells from cells farther back, where the concentration is not as great. Eventually these cells draw water osmotically from the xylem strands. The source of energy for this process of concentration of leaf solutions is the heat of the surrounding atmosphere, which causes the aqueous molecules to break away from the films covering the parenchyma walls next to the air chambers.

(2) *Water pores and nectaries.*— Water pores and nectaries are curious instances of the passage of liquid water out of the plant body. No completely satisfactory explanation of these occurrences has yet been given. Osmotic action surely plays an important part here, but as yet no accurate means of determining the exact process has been devised. The difficulty lies in the fact that in these cells the movement of the water is in the opposite direction from that which would be expected from the principles of osmotic action. The following hypothesis may help to bring the facts together :

The modified cells bordering a water pore are perhaps irritable in a peculiar way. It may be that the protoplasmic

sac, upon being stretched beyond a certain limit, changes its nature in some way so as to become permeable to the contained solutes. If this were the case, turgidity would rise to the critical point, and then, when the change in permeability took place, there would result an exudation of cell sap through the plasmic membrane, the contraction of the previously stretched cellulose wall forcing the solution through the now permeable layers. This exudation might be equal in all directions, or might take place mainly in the direction of the water pore, as if the portion of the protoplast lying next the modified air cavity were the only part to become permeable at the assumed critical point. It seems probable that, if such an occurrence takes place at all, the change is brought about uniformly, and that the exudation from the vacuole is equal in all directions. But, since the adjacent tissues of the leaf are engorged with water, a marked flow could take place only in the free direction, namely, outward into the cavity of the pore itself.

After the cellulose walls had ceased to contract, external pressure would be removed from the protoplast, the flow of liquid would cease, the internal pressure would have fallen below that at the assumed critical point, and it is not at all inconceivable that under these conditions the protoplasm might return to its original condition of semi-permeability toward the contained solution. If this were so, absorption from the surrounding tissues would again take place, and turgidity would gradually return until the critical point was again reached, when the process of external discharge would again occur. Evaporation from the surface of the exuded droplet, which must become rapid as soon as the latter is pressed into the air space, would concentrate the solution, and thus osmotically prevent resorption of the extruded water. More than that, with the increasing concentration it would act as a plasmolyzing solution to extract still more water from

within. Experiments are needed to determine whether or not this sort of extraction of water does really take place in water pores. At any rate, the important point lies here, that there *is* an original exudation of cell sap through the protoplasm. That in some cases at least the exudation is truly a portion of the sap, and not pure water, has been shown by Bonnier[1] in the case of honey-dew, and by Dandeno[2] in the case of guttation drops. Also Moll[3] showed that when shoots whose leaves bore water pores were injected from below with the juice of Phytolacca decandra, the exudation always contained the color. Since water cannot pass from the xylem to the outside without traversing the cells bordering upon the pore, the exuded water bearing the coloring matter must have pased through these cells.

The only serious difficulty with this hypothesis as an explanation of the action of water pores lies in the assumed continually decreasing concentration of the cell sap. But there is good evidence that the protoplasm of the plant cell is continually discharging substances into the vacuole, which increase the osmotic pressure therein. There is apparently no reason why we may not postulate this same property, perhaps in an unusually marked degree, in the case of the cells just discussed.

In the case of nectaries there is exhibited an apparently similar set of phenomena. Wilson's work[4] shows that after the dissolved substances of the nectar (mainly sugar) have once passed out of the cells and into the cup of the nectary,

[1] G. BONNIER, "Recherches expérimentelles sur la miellée," *Rev. gen. bot.*, Vol. VIII (1896), pp. 1–22.

[2] J. B. DANDENO, "An Investigation into the Effects of Water, etc., on Foliage Leaves," *Trans. Canad. Inst.*, Vol. VII (1901), pp. 238–350.

[3] J. W. MOLL, "Untersuchungen über Tropfenausscheidung u. Injection von Blättern," *Verslagen en Mededeel. d. k. Akad. v. Wetensch. te Amsterdam*, Vol. XV (1880), pp. 237–337.

[4] W. P. WILSON, "The Cause of the Excretion of Water on the Surface of Nectaries," *Unters. aus d. bot. Inst. zu Tübingen*, Vol. I (1881), pp. 1–22.

the high osmotic pressure caused by evaporation will fully account for the outward passage of water which keeps the nectar of flowers and leaves in the liquid condition on the dryest days. Nectaries may even be artificially formed in this way ; if minute granules of sugar are placed upon the hyphæ of Mucor, droplets of water will soon form and increase in size until they run down and fall off. The same may be done with leaves ; a small mass of sugar upon the epidermis will soon extract enough water osmotically to make a large droplet. The method of extraction of the water in these artificial cases is clear enough after a small portion of the sugar has been put into solution. Water to form the first minute amount of solution cannot be withdrawn from the leaf-cells by osmotic action. But all cellulose membranes, even cuticularized ones, are more or less saturated with water of imbibition. Now, the sugar particles resting upon the leaf come in contact with these moist membranes and immediately begin to dissolve in the water therein imbibed. After the start is once made, be it ever so infinitesimal, osmotic action will accomplish the outward flow of water from the comparatively weak solution within the cells to the saturated one on the surface.

But in the case of the natural nectary the original exudation of the sugar-containing sap has not yet been accounted for. Perhaps this can be explained in a manner parallel to that just postulated for the case of the water pore. At a certain stage in its development the glandular tissue of the nectary may undergo a change such that, through a rapid increase in soluble content, the osmotic pressure of the sap of its component cells rises suddenly. This rise in turgor pressure may act as a stimulus upon the protoplasm, and the latter may, in turn, respond by a change in its structure, so that it becomes permeable to solutes as well as water. If this be true, the contraction of the stretched cellulose walls must

press the cell sap out of the numerous cells of the gland, and this may accumulate on the external surface. Owing to evaporation this droplet of exuded sap must immediately begin to increase in concentration, and from that time on Wilson's observations are sufficient to explain the maintenance of liquid in the nectary.

It has been shown,[1] however, in certain cases, that if the nectar be removed, new nectar will be secreted. According to the above hypothesis, this must be due to another increase of turgidity and another exudation of cell sap. Thus it must be supposed that, after the first discharge, the conditions again come into equilibrium, and the plasmic membranes again become semi-permeable, only to repeat the former process of excretion if the nectar is again removed. Perhaps the evaporating, and therefore concentrated, solution on the surface acts as a plasmolyzing agent, keeping the turgidity of the gland cells down by extraction of water. If this were so, the removal of the nectar might easily cause an increase in turgidity, which might, in turn, bring about the response of exudation.

Wilson found the shining droplets of water which occur on certain molds to be of the same nature as the artificial droplets which he was able to produce by sprinkling the hyphæ with pulverized sugar. When these natural droplets are removed they again return, but this does not occur if the hyphae are carefully washed with distilled water. Careful examination of the places where droplets had been removed without washing showed minute crystals of sugar upon the surface. Thus, if the air becomes dry, evaporation may become so great that the droplets disappear, but as soon as evaporation is checked, the crystals of sugar lying upon the surface go into solution in the imbibed water of

[1] H. HAUPT, "Zur Secretionsmechanik der extrafloralen Nektarien," *Flora*, Vol. XC (1902), pp. 1-41. On the general subject of secretion, see W. PFEFFER, *Studien zur Energetik der Pflanzen*, Leipzig, 1892.

the cell wall and cause a renewed outward flow of water, and the renewal of the droplet.

The recent work of Haupt on extra-floral nectaries adds to our knowledge of gland action. This author finds that the secretion of sugar in these nectaries begins only when the gland has attained a certain stage of development, and then only when transpiration is relatively slight. This makes it appear as though the protoplasmic layers become permeable to sugar at a certain phase in the series of developmental changes, providing there be at that time a great accumulation of water in the cells. This last provision means that the cell walls and plasmic membranes are strongly stretched. After secretion has begun an increase in humidity causes an increase in the excretion of water from the gland, but that of sugar remains constant. Hence it may be concluded that the humidity, *i. e.*, the amount of water evaporated, has no direct effect upon the permeability of the protoplast to sugar. For the beginning of the secretion, Haupt finds that a certain minimum temperature is necessary. The temperature very probably affects the physical nature of the protoplasmic layer. That the red-yellow portion of the solar spectrum influences the activity of these glands in a profound manner has been already mentioned.[1]

It is important to note in this connection that, while protoplasm is generally to be regarded as semi-permeable toward many solutes, yet there is evidence from almost all parts of the plant kingdom that it is often permeable to such substances as sugar, acids, inorganic salts, etc. The penetration of these substances is usually a slow process, however. Now the variation in the permeability of different protoplasts, even of the same plant, may be taken as evidence that slight differences in the protoplasm may cause rather great differences in permeability. This makes the

[1] See p. 78.

alteration in permeability which is postulated above in reference to water pores and nectaries less difficult of supposition than it might appear at first thought. Also, the variations in permeability which are known to occur in certain cells, *e.g.*, those which can be brought about in almost any cell by chemical agents like $H_g\ Cl_2$, and by change in temperature, for example the cold plasmolysis of Spirogyra, render it at least possible that excess of turgor pressure may alter permeability. Indeed, a similar phenomenon to the one postulated was observed by Oltmanns[1] in the case of Fucus, and it is more than probable that the exudations from certain "sensitive" pulvini often, if not always, contain solutes.

(3) *Exudation.*—If the stem of a plant be severed near its base and a mercury manometer be attached to the portion connected with the roots, an exudation of water under pressure may often be demonstrated. This so-called exudation pressure is not uniform, but varies in an irregular manner, sometimes sinking below the zero point of the scale and at other times amounting to an atmosphere or more. A similar pressure is reported in various parts of the plant, even high up in the tops of trees, as was noted by Molisch[2] in the case of Cocos and Arengo. A series of experiments upon this subject was performed by Wieler,[3] who came to the conclusion that the power of exuding sap is a general one among plant cells. Other experiments which are not nearly so satisfactory are those made by Kraus[4] and

[1] F. OLTMANNS, "Ueber d. Bedeutung der Koncentrationsänderung des Meerwassers für d. Leben d. Algen," *Sitzungsber. d. k. preuss. Akad. d. Wiss. zu Berlin*, Jahrg. 1891, p. 183.

[2] H. MOLISCH, "Die Secretion des Palmenweins und ihre Ursachen," *Oesterr. bot. Zeitschr.*, Vol. XLIX (1899), p. 74. Also see W. FIGDOR, "Untersuchungen über d. Erscheinung des Blutungsdruckes in den Tropen," *Sitzungsber. d. kais. Akad. d. Wiss. zu Wien*, math.-nat. hist. Klasse, Vol. CVII (1898), p. 640. This is noted in *Oesterr. bot. Zeitschr.*, Vol. XLVIII (1898), pp. 359, 360.

[3] A. WIELER, "Das Bluten der Pflanzen," *Cohns Beiträge*, Vol. VI (1893), pp. 1-211.

[4] C. KRAUS, "Untersuchungen über den Säftedruck der Pflanzen," *Flora*, Vol. XL (1882), pp. 2 ff.

Pitra.[1] The former found that various parts of stems and roots exuded water when cut out from the plant and placed partly submerged in wet sand. Details of the experiments are not given, and there are many circumstances in the somewhat superficial account which lead the reader to doubt whether the author was dealing with true bleeding; for there are other factors, such as the expansion of gases in the wood, which may cause exudation under certain conditions. Pitra's experiments are much more convincing than those of Kraus, but it is still somewhat doubtful whether very much true bleeding was observed by either of these authors. Pitra makes one observation which is of interest here, however. If a cut shoot be inverted with its leaves under water and its stem in air, bleeding from the cut surface of the stem will ensue. That is, if leaves are placed in a position to absorb water, they can do so in a manner entirely similar to that exhibited by roots, and a *leaf-pressure* corresponding to the normal root-pressure seems to be developed. The observations of Pitra in this regard have been substantiated by Molisch,[2] who finds the same to hold true if the leaves are not placed in water but are surrounded by moist air.

The evidence seems to be good that bleeding may occur (1) in the case of cut stumps to which active roots are still attached; (2) at the cut surface where the crown or inflorescence has been removed from certain palms (Molisch), and (3) at the cut surface of certain stems whose leaves are submerged in water (Pitra). It may occur in other parts, but it seems that true bleeding has not been unquestionably demonstrated elsewhere in tall plants.

Exudation pressure has often been ascribed to osmotic

[1] A. PITRA, "Versuche über d. Druckkraft d. Stammorgane bei d. Erscheinungen des Blutens u. Thränens," *Jahrb. f. wiss. Bot.*, Vol. XI (1877), pp. 437–530.

[2] H. MOLISCH, "Ueber localen Blutungsdruck und seine Ursachen," *Bot. Zeitg.*, Vol. LX (1902), pp. 45–63.

phenomena occurring within the cells.[1] If this be the true explanation, we have no exact knowledge of the processes by which these phenomena occur. That osmotic pressure within the vacuoles might cause movement of an exuded solution under pressure, through intercellular spaces or through the channels of the xylem, need not be questioned; this can be simulated in the laboratory with the ordinary thistle tube of molasses closed with animal membrane. But to explain the phenomenon of exudation pressure it must be shown how it comes about that this solution gets into the channels at all. A small amount of solution might be exuded in a manner similar to that supposed above in connection with nectaries and water pores. But the absence of evaporation within the plant body will deprive us of that source of energy to which has been ascribed the maintenance of a relatively high concentration in the exudate of nectaries. However, it is probable that even in nectaries and water pores this discovery of Wilson is not of fundamental importance. The main desideratum is to know how the original exudate comes to get through the otherwise only semipermeable protoplasm; if a little can be exuded there seems to be no reason why more could not pass out in the same way. In all the cases of exudation the exudate is known to be a solution; the solutes of the vacuoles pass out and appear in the exudate. This is an unquestionable indication that the protoplasmic membrane is permeable to them.

An explanation of this phenomenon was elaborated by Pfeffer[2] and later, apparently independently, by Fuchs.[3] This depends upon the assumption of some sort of vital activity within the cells. These authors point out that in order to have a current of water through a cell, it is only

[1] W. PFEFFER, *Osmotische Untersuchungen*, Leipzig, 1877, p. 223.

[2] *Ibid.*, pp. 222–5.

[3] K. FUCHS, "Zur Theorie der Bewegung des Wassers im lebenden Pflanzenkörper," *Beih. Bot. Centralbl.*, Vol. X (1901), pp. 305–8.

necessary that the concentration of the cell sap at the point of entrance be higher than at the point of exit. If such a condition could be maintained, a slight movement of water would undoubtedly take place, though in the writer's judgment it would be very inadequate in amount. But the insurmountable difficulty in this explanation is the continuous maintenance of this difference of concentration in different parts of the same cell. This could only be accomplished by the active absorption and precipitation or fixation of the active osmotic substances in one region of the protoplast, and their secretion and solution in another part. Such a supposition in its simplest form involves the carrying back of these substances by the protoplasm, for example, from one end of the cell to the other, with as great rapidity as they can diffuse in the opposite direction through the cell sap. This could only occur with enormous expenditure of energy on the part of the protoplasm, and it is difficult to imagine any adequate source for this energy.

Still another proposed explanation of the passage of water through a cell in any given direction has been offered by Pfeffer.[1] He points out that, if the membrane where the water enters be less permeable to solutes than that where it escapes, a continuous flow will take place. This is undoubtedly true, but the flow would be still more marked if the second membrane were removed altogether; for it can act only as an obstruction to the upward expansion of the inclosed liquid as water is taken in through the semi-permeable membrane below. The resulting system is such as would be obtained if the thistle tube osmometer used for illustration of osmotic pressure were to have its stem plugged with cotton. The cotton would hinder the rise of the liquid column, but would not stop it altogether. Copeland[2] has actually constructed a

[1] W. Pfeffer, *Osmotische Untersuchungen*, Leipzig, 1877, p. 225.

[2] E. B. Copeland, "An Artificial Endodermis Cell," *Bot. Gaz.*, Vol. XXIX (1900), pp. 437–9.

piece of apparatus by which a current is maintained through a cell, different parts of whose wall are unequally permeable.

That exudation pressure depends upon vital activity seems evident from the fact that it ceases with death. Another line of evidence which points toward the necessity of active protoplasts for exudation is that brought forward by Wieler,[1] when he records that, if a bleeding plant is deprived of oxygen, exudation stops. He observed the same result when the plant was anæsthetized with chloroform. The exuded liquid varies in its concentration in different plants, usually becoming weaker as bleeding continues, but there seems to be no discovered relation between the exudation pressure and the concentration of the exudate.[2]

The whole subject of exudation and sap pressure is viewed in an entirely new light by Molisch[3] in his last paper on these phenomena. He presents convincing evidence that in all cases where true bleeding has been observed it is a phenomenon connected with the stimulus of wounding or with the formation of new tissue, such as callus, over the wound surface. Thus, the method of decapitation and of boring, for the study of exudation pressure, becomes at least of very doubtful use in the investigation of the normal pressure within the plant. It is impossible to insert a manometer into a stem without making a wound, and, according to Molisch's conclusions, this wound itself is sufficient to cause a pathological condition of the neighboring tissue such that exudation ensues. In the light of these considerations, then, it seems extremely doubtful whether there is exhibited in the normal, uninjured plant any such phenomenon as that of sap pressure. Where exudation occurs in the pathological wound tissues it must be due to some such change in permeability of the protoplasts as was postulated above.

[1] A. WIELER, "Das Bluten der Pflanzen," *Cohns Beiträge*, Vol. VI (1893), p. 158.
[2] *Ibid.*, pp. 65, 69.
[3] H. MOLISCH, "Ueber localen Blutungsdruck und seine Ursachen," *Bot. Zeitg.*, Vol. LX (1902), pp. 45–63.

(4) *Summary of water loss.* — By evaporation pure water is lost from the plant. Thus the osmotic pressure of the fluids in the leaves and near evaporating surfaces is increased and other water diffuses to these regions, thus tending to re-establish an equilibrium of diffusion tension. Water is eventually taken from the xylem vessels and, since these are not lined with protoplasm, a mass movement of water is set up, flowing upward through the xylem strands. This carries with it whatever dissolved substances have been extruded into these strands from the roots. Evaporation cools the leaves. Leaves, etc., are able to extrude solution upon their surface through specialized openings, the glands and water pores. The exact process by which this extrusion takes place is not known; it is probably an osmotic one, perhaps coupled with some periodic change in permeability of the protoplasts. Leaf cells will act in the same way in the opposite direction without regard to water pores (Pitra, Molisch).

Root cells are able to take in water and solutes and then to pass them on into the xylem. It seems that sap pressure, whether in roots or in wound tissue, must be explained along the same general lines as the action of glands and water pores. Whether a periodic external exudation occurs in roots is not known, but it seems not improbable that at some times roots may let out solutes. Indeed, Czapek[1] and Molisch[2] have observed extrusions from root hairs which are not unlike those from water pores. It may be that some sort of a periodic extrusion of solutes is a fundamental property of protoplasm. If this be true, it is a phenomenon not unlike that exhibited in pulsating vacuoles.

b) The upward movement of water in trees and other tall plants. — Owing to almost insurmountable difficulties in

[1] F. Czapek, "Zur Lehre von den Wurzelausscheidungen," *Jahrb. f. wiss. Bot.*, Vol. XXIX (1896), pp. 321-90.

[2] H. Molisch, "Ueber Wurzelausscheidungen und deren Einwirkung auf organische Substanzen," *Sitzungsber. d. kais. Akad. d. Wiss. zu Wien*, math.-nat. hist. Klasse, Vol. XCVI (1887), pp. 84-109.

experimentation, an exact knowledge of the manner in which water is raised in tall stems has not yet been reached. Various hypothetical explanations of the observed phenomena have been offered, but no one of them has been thoroughly established. Imbibition, capillarity, the lifting power of evaporation exerted upon a cohering water column, physical osmosis, and undefined "vital activity," have all been invoked to explain the phenomena of the ascent of sap in trees. It is not intended to take up here the discussion of any of these hypotheses excepting those which deal with osmotic pressure and diffusion.

It is well known that the xylem is the conducting region for water. Since the tracheæ, which mainly compose it, are dead and contain no protoplasmic lining, there cannot be attributed to them any *active* part in lifting the water which they contain. It has been proposed[1] as a partial explanation of this rise of water that the exudation pressure which is made externally apparent where a plant is cut or broken may be normally active within the xylem and may thus furnish a part of the needed force. This is a very plausible theory if not pushed too far.

It can hardly be supposed that if this pressure is concerned in raising water in the xylem it is exclusively applied at the base of the stem or in the roots. It is much easier to suppose that the various groups of cells exerting exudation pressure act as a set of relay pumps, each group taking the

[1] This theory, as far as I know, was first clearly put by GODLEWSKI in his paper entitled "Zur Theorie d. Wasserbewegung in den Pflanzen," *Jahrb. f. wiss. Bot.*, Vol. XV (1884), pp. 569–630. WESTERMAIER had somewhat the same idea a year previous to this, but did not develop it as well. His article is "Zur Kenntniss der osmotischen Leistungen des lebenden Parenchyms," *Ber. d. deutsch. bot. Ges.*, Vol. I (1883), pp. 371–81. But the best exponent of the pumping action of parenchyma was JANSE, whose ideas are expressed in a paper entitled "Die Mitwirkung des Markstrahlen bei der Wasserbewegung im Holze," *Jahrb. f. wiss. Bot.*, Vol. XVIII (1887), pp. 1–69. The last-named author elaborated Godlewski's theory and supported it with experiment. Many more citations might be made; the literature is very voluminous. For a very complete discussion of this subject, see E. B. COPELAND, "The Rise of the Transpiration Stream," *Bot. Gaz.*, Vol. XXXIV (1902), pp. 161–93, 260–83.

sap from the adjacent tracheæ and passing it on upwards. But if sap has already passed through a set of these active cells in a manner similar to that described in connection with water pores, its concentration cannot be lower than that of the cell sap of these cells when they are stretched to their utmost; indeed, from the loss of pure water to other cells along its path its concentration is apt to be even higher. If this same sap, now in the tracheæ, is to enter another set of such active cells and be pressed still higher up, the sap of the latter must necessarily possess a higher osmotic concentration than the fluid to be absorbed; and after it has been pressed out of them into tracheæ still farther up the stem, it must have gained in concentration. Thus any such explanation of the rise of sap in stems involves a gradually increasing concentration of the sap as it passes upward. There seems to be no experimental evidence of this as a fact. It is true that the sap in leaves is more concentrated than that in the stem, but there seem to have been put on record no observations of a *gradual* increase in concentration toward the summit of a tree. Evaporation from the leaves would account for the observed fact.

The above presentation will stand for various hypotheses which have been proposed in this regard, all involving some sort of a periodic variation in permeability. Godlewski and Janse have attempted to locate the active cells in the cortex or medullary rays of woody stems, but these attempts have apparently failed. In fact, the whole idea that the ascent of sap in tall stems has any necessary dependence upon the presence of living cells may be doubted very much on the following experimental grounds:

In his study of bleeding Wieler[1] came to the conclusion that this process, while it is probably a general property of

[1] A. WIELER, "Das Bluten der Pflanzen," *Cohns Beiträge*, Vol. VI (1893), pp. 1-211.

protoplasm, yet plays no leading rôle in the lifting of water up tall stems. As far back as 1853 T. Hartig[1] showed that a poison (ferric pyrolignate) would pass up the stem of a tree for over 12 meters. He bored five radial auger-holes in the tree trunk near its base, all meeting at the center. These holes were filled with the poison solution and then plugged. When the tree was cut down, the star-shaped stain of the poison was found in a cross-section over 12 meters above the holes. Strasburger[2] performed the same experiment more thoroughly. Trees were cut off and set into tubs of poison, such as aqueous solutions of $CuSO_4$ and picric acid. The poison ascended to the leaves, a distance of twenty-one meters in the tallest tree. Of course, if these violent protoplasmic poisons ascend the trunk, they must kill all cells lying in their path. Therefore the living cells of the stems cannot be necessary for the rise of sap.

But after the leaves had been killed the stem ceased to absorb more solution, or absorption took place very slowly. This may be explained by the fact that most leaves collapse and dry upon being killed. The cause of the rise of sap is perhaps the evaporation from the surface of the leaves, and in order that it should rise the leaves must be in their normal turgid condition. Evaporation may thus result in concentration of the solution on the surface of the walls of the parenchyma, thus causing an outward osmotic flow of water from the cells, the solutions of which in turn become more concentrated and extract water from cells lying still farther within the plant. This process may be thought of as continuing until water is finally extracted from the zylem. Here the osmotic withdrawal of water would probably become

[1] T. HARTIG, "Ueber die endosmotischen Eigenschaften der Pflanzenhäute," *Bot. Zeitg.*, Vol. XI (1853), pp. 309–17.

[2] E. STRASBURGER, *Ueber den Bau und die Verrichtungen der Leitungsbahnen in den Pflanzen*, Jena, 1891; IDEM, "Ueber das Saftsteigen," *Histologische Beiträge*, Vol. V, Jena, 1893.

a mechanical tension upon the minute films and columns reaching to the foot of the tree. Strasburger's experiments seem to show that atmospheric pressure cannot play a part here; for his tallest tree was as high as a column of water which would balance a pressure of two atmospheres. However, it is to be remembered that the water in a tree trunk is not in continuous columns, but that the columns are divided by air bubbles.

That the leaves play the part just ascribed to them is practically proved. Besides the experiments of Strasburger may be cited that of Dixon,[1] wherein he showed that when the leaves at the top of a tall shoot were killed, the upward passage of water was checked, even though the stem were still uninjured.

The hypothesis that evaporation is the source of the energy required in raising water dates back, in its general form, to Dutrochet.[2] The main points of this idea are the entrance of the water below and its evaporation above. The point which has caused most trouble lies in the lack of proof that water columns such as are found in the tree have cohesion enough to be drawn up by evaporation to a height far exceeding that to which this liquid would be supported by one atmosphere. That it will cohere somewhat beyond the height to balance a pressure of one atmosphere has been shown by Böhm,[3] Askenasy,[4] and Copeland.[5] The former of these showed that evaporation from the surface of a twig of Thuya attached to an upright tube of water, the lower end

[1] H. H. DIXON, "Note on the Rôle of Osmosis in Transpiration," *Proceed. Roy. Irish Acad.*, Sec. III, Vol. III (1898), pp. 767–75.

[2] M. H. DUTROCHET, *Mémoires pour servir à l'histoire anatomique et physiologique des végétaux et des animaux*, Brussels, 1837.

[3] J. BÖHM, "Capillarität und Saftsteigen," *Ber. d. deutsch. bot. Ges.*, Vol. XI (1893), pp. 203–12.

[4] E. ASKANASY, "Beiträge zur Erklärung des Saftsteigens," *Verhandl. d. naturl.-med. Verein zu Heidelberg*, N. F., Vol. V (1896), pp. 429–48.

[5] E. B. COPELAND, "The Rise of the Transpiration Stream," *Bot. Gaz.*, Vol. XXXIV (1902), pp. 161–93, 260–83.

of which dipped in mercury, was sufficient to lift a column of the latter liquid higher than the barometer column at the time of the experiment. Askenasy was able to construct apparatus whereby he could demonstrate a pressure of 90 cm. of mercury arising from evaporation of water from a saturated plaster of Paris plate. Copeland constructed a column of plaster of Paris 3 mm. in diameter and 12.4 m. high, which terminated below in a mercury manometer and above in a $Cu_2Fe(CN)_6$ membrane. The whole column was as nearly saturated with water as might be (there were many air-bubbles, however), and the membrane above was covered by an exposed solution of $CuSO_4$. Evaporation from the surface of this solution caused, in five days, a suction on the manometer below of 301 mm. of mercury. Essentially this apparatus is a bundle of minute water columns held in the pores of the plaster, but broken here and there by air bubbles. There was certainly enough water in the column to give a pressure of more than 459 mm. of mercury (1 atmosphere *minus* 301 mm.). If this be true, the suction set up by evaporation above surpassed the pressure of an atmosphere.

This theory of sap ascent seems to be gaining ground, and it is quite probable that the idea of Godlewski and Janse will eventually be put entirely aside. The experiments of Strasburger and Dixon show that osmotic pressure must be active in the leaves in order that sap may ascend at its usual rate. Another proof that living protoplasm is necessary in leaves lies in the fact that transpiration can be influenced by anæsthetics.[1] Such reagents act in a similar manner to the

[1] For a discussion of the relation of anæsthetics to transpiration see H. H. DIXON, "On the Effects of Stimulation and Anæsthetic Gases on Transpiration," *Proceed. Roy. Irish Acad.*, Ser. III, Vol. IV (1898), pp. 618–26; H. JUMELLE, "Influence des anesthétiques sur la transpiration chlorophyllienne," *Rev. gen. bot.*, Vol. II (1890), pp. 417–32; IDEM, "Nouvelles recherches sur l'assimilation et la transpiration chlorophylliennes," *ibid.*, Vol. III (1891), pp. 241–88, 293–305; A. SCHNEIDER, "The Influence of Anæsthetics on Plant Transpiration," *Bot. Gaz.*, Vol. XVIII (1893), pp. 57–69; A. WOODS, "Some Recent Investigations on the Evaporation of Water from Plants," *ibid.*, Vol. XVIII (1893), p. 304-10.

poisons used by Strasburger, though, of course, in not so marked a manner. They probably cause partial plasmolysis in the leaf cells and thus disturb diffusion and evaporation. Still another point which may be construed in this same manner is the observation of Kossaroff[1] that an increase in the amount of CO_2 in the water in which are placed cut twigs, with and without leaves, is accompanied by a marked falling off in water absorption. What can be the reason for this we are unable even to conjecture, but it certainly appears as though the chemical nature of the membranes were involved. It cannot be due to the osmotic pressure of the dissolved CO_2, since this gas penetrates all protoplasts with the utmost ease. Possibly the CO_2 may precipitate in the wood and form bubbles which plug the water channels; but this, too, seems unlikely. It seems more probable that the dissolved gas affects the protoplasmic membranes in the leaves, causing some change in their osmotic properties.

The whole problem of water ascent remains a puzzling one, one which must wait for solution until the development of better and more exact methods of experimentation. In the meantime it will probably be more profitable to devote attention to some of the more definite and restricted problems of the cell itself. In the present condition of our knowledge of plasmic membranes, for example, it is almost foolhardy to attempt to settle such a complex question as the one just briefly reviewed; but once knowing the nature of these plasmic membranes, it is not improbable that the solution of the problem of water transport will follow as the simplest corollary.

III. SUMMARY OF THE CHAPTER

In general, the process of water absorption and water movement may be stated as follows: The imbibed water of the cell walls, the water of the protoplasm itself, and the

[1] P. KOSSAROFF, "Die Wirkung der Kohlensäure auf den Wassertransport in den Pflanzen," *Bot. Centralbl.*, Vol. LXXXIII (1900), pp. 138-44.

water of the substratum in which the organism is growing, are to be regarded as one continuous mass of liquid. Thus, if the diffusion tension of water in any part of the plant becomes less than it is at any other point, diffusion takes place and equilibrium is restored. In the same way, if the diffusion tension within the plant falls below that of the substratum, diffusion of water into the plant must immediately occur.

This process of simple diffusion is sufficient to account for absorption and for transport in the simple plant bodies and in any small portion of larger bodies. But in the more complex bodies of higher plants this is not sufficient. Just how the sap is raised in trees is not surely known. There are at present two main theories to account for it: (1) It is supposed to be raised by periodic pumping action of living cells in the trunk. (2) It is supposed that evaporation and the resulting osmotic concentration in the leaves will draw it up from the roots, the cohesion of the minute water columns being supposed to be of sufficient magnitude to prevent their being broken by the strain.

CHAPTER III

ABSORPTION AND TRANSMISSION OF SOLUTES

With the exception of the naked amœboid cells occurring in certain stages of the life histories of a few fungi and algæ, together with the cell complexes constituting Myxomycete plasmodia, plant cells are unable to engulf solid food. The presence of the cellulose membrane makes this fact very evident. In order to be absorbed into a cell, any substance must first be in the form of an aqueous solution. Even where the process of engulfing takes place, the food does not truly pass *inside* the protoplasmic body until it is dissolved; around each food body in a plasmodium is a vacuole lined by a plasmic membrane which is probably identical in nature and origin with that covering the exterior of the protoplasmic mass. In such cases the food is digested in this vacuole and the products of digestion are absorbed through this membrane.

I. ABSORPTION OF GASES

There are, in general, two forms of material in solution which are absorbed by the plant, namely, gases and solids. As has been seen, gases enter into aqueous solution when they are simply brought into contact with the solvent. All the natural water on the surface of the earth contains in solution oxygen, nitrogen, argon, carbon dioxid, etc. The first and the last of these gases are the only ones which are important in plant metabolism. The moist cellulose membrane and the protoplasts are all permeable to these dissolved gases, and, being soluble in water, they will diffuse wherever it can diffuse. Thus there must be a tendency to equalize the diffusion tension of oxygen and carbon dioxid

(and of the other two gases also, though they are not used in metabolism) throughout the water in the substratum and that contained within the plant. And, since these two masses of water are continuous, simple diffusion will account for the exchanges which take place between the dissolved gases of the soil and those of the roots of the land plant. The same sort of diffusion takes place between the internal solution of the water plant and the surrounding water. Not only may absorption thus take place, but also the giving off of gaseous waste products. In the case of aquatic saprophytes and parasites, oxygen is absorbed and carbon dioxid given off. In that of aquatic green plants this process occurs in darkness, but in light, carbon dioxid is absorbed, while oxygen is given off. The roots of land plants are always absorbing oxygen and eliminating carbon dioxid.

But by far the greater portion of the gaseous exchange in land plants and semi-aquatics takes place, not through the soil water, but through the wet membranes which are in contact with the air. This is especially so in green plants, whose leaves are peculiarly constructed so as to expose moist cellulose membranes in air chambers which are in connection with the outer air through stomata.[1] By a number of researches[2] it has been shown that dry walls are but slightly, if at all, permeable to gases and that the more moist they are the more readily are they permeable.

[1] F. F. BLACKMAN, "Experimental Researches on Vegetable Assimilation and Respiration": II, "On the Paths of Gaseous Exchange between Aerial Leaves and the Atmosphere," *Phil. Trans. Roy. Soc.*, London, B., Vol. CLXXXVI (1895), pp. 503–62; H. T. BROWNE and F. ESCOMBE, "Static Diffusion of Gases and Liquids in Relation to the Assimilation of Carbon and Translocation in Plants," *ibid.*, Vol. CXCIII (1900), pp. 223–92.

[2] N. J. C. MÜLLER, "Untersuchungen über d. Diffusion atmosphärischer Gase in der Pflanze und der Gasausscheidung unter verschiedenen Beleuchtungsbedingungen," *Jahrb. f. wiss. Bot.*, Vol. VII (1869), pp. 143–92; E. LIETZMANN, "Ueber die Permeabilität vegetabilischer Zellmembranen in Bezug auf atmosphärische Luft," *Flora*, Vol. LXX (1887), pp. 339–86; J. WIESNER and H. MOLISCH, "Untersuchungen über die Gasbewegung in der Pflanze," *Sitzungsber. d. kais. Akad. d. Wiss. zu Wien* math.-nat. hist. Klasse, Vol. XCVIII (1889), Abth. 1; P. CLAUSEN, "Ueber die Durchlässigkeit der Tracheidenwände für atmosphärische Luft," *Flora*, Vol. LXXXVIII (1901), pp. 422–69.

During the hours of sunlight, when the process of photosynthesis is going on, carbon dioxid is being combined with water to produce carbohydrates within the chlorophyll-bearing cells. Thus, at this time the diffusion tension of this gas in the solutions of these cells is much lower than it is in the outer air and in the air chambers. Therefore, there must be a constant diffusion stream of carbon dioxid moving through the stomata into the air chambers, going into solution in the imbibed water of the moist cell walls wherever it touches them, and diffusing as a solute through the tissues of the plant to the places of low diffusion tension.

The oxygen which is given off in photosynthesis finds its way to the outer air in the same manner as that by which the carbon dioxid enters. The oxygen tension becomes higher in the green cells than in the outer air, and a diffusion stream of this gas is at once set up in the direction of the air chamber, where it goes out of solution and then diffuses as a gas through the stomata into the outer air. During the night when photosynthesis has ceased this oxygen stream slackens and stops, as does also the incoming stream of carbon dioxid. What oxygen is used in respiration at this time enters from the outer air, and the carbon dioxid produced by this process finds its way out in a manner exactly similar to that in which the other gas escapes during the day.

Epidermal tissues of leaves and stems are also imbibed with water, but the amount of water which they can hold is comparatively small on account of the fact that the external walls are heavily impregnated with waxy substances. Hence, as would be expected, a small amount of gaseous exchange between the atmosphere and the plant takes place directly through these membranes.[1]

[1] F. F. Blackman, "Experimental Researches," etc.: II, "On the Paths of Gaseous Exchange between Aerial Leaves and the Atmosphere," *Phil. Trans. Roy. Soc.*, London, B., Vol. CLXXXVI (1895), pp. 503–62.

II. ABSORPTION OF DISSOLVED SOLIDS AND LIQUIDS

It is probable that the protoplasmic membranes of plant cells are, normally at least, slightly permeable to all substances which the organism needs to absorb. We have direct evidence of the permeability of protoplasm to many of these substances; this evidence was presented in chap. i of this Part. How it comes about that a cell may retain turgor and still be permeable to solutes was also discussed there. The substances which are diffusing into a plant cell at any moment cannot be the ones which are producing the turgor pressure. While certain organic molecules are maintaining the turgor, for instance, many inorganic ions may be diffusing into the cell, because the partial diffusion tension due to them is lower within than it is without. It is also possible that permeability changes from time to time, so that a substance which cannot penetrate the protoplast at one time may do so at another. Turgidity is maintained, and the protoplasmic layer kept stretched and in contact with the imbibed cellulose walls, by the osmotic pressure of certain substances which are probably formed within the cell and to which the protoplast is but slightly permeable.[1]

Thus it is clear that the absorption of solid and liquid solutes from the surrounding solution is, like that of gases, merely a phenomenon of diffusion, the particles moving toward that part of the solution where lowest diffusion tension of that substance obtains. Water plants possibly absorb solutes through all parts of their submerged surfaces. Land plants can absorb them only where they are in contact with the moist substratum, mainly through the roots and root hairs.

The so-called power of selection of absorbing organs deserves some attention here. It is observed that some plants absorb much more of certain substances than others,

[1] O. H. VON MAYENBURG, "Lösungsconcentration und Turgorregulation bei den Schimmelpilzen," *Jahrb. f. wiss. Bot.*, Vol. XXXVI (1901), pp. 381–420.

and that different plants absorb different amounts of the same substance. Failure to absorb a solute which is plentiful in the external solution may be due to either one of two causes : either the protoplasmic membrane is impermeable to that substance, or its diffusion tension within and without are equal. If a substance is not used in metabolism, it may simply remain in the cell sap, at the same concentration as is the surrounding medium, or it may be precipitated or condensed in the cell sap or in the protoplasm, and thus continue to accumulate. An example of this last possibility is met with in the case of the storage of carbohydrates. Sugar diffuses into a cell and is there polymerized into insoluble starch. This process continually removes the sugar from solution, so that inward diffusion must continue as long as starch can be formed, and as long as sugar is plentiful outside. Another example of accumulation is the case cited by MacDougal, in which large quantities of metallic copper were found in the cells of an oak tree (see p. 69).

If the substance is used in metabolism, as are NO_3 ions, for instance, there must also occur a continuous diminution of the internal diffusion tension of these particles, which can only be met by inward diffusion from without. If a substance is being rapidly used, this inward diffusion will be correspondingly rapid; if it is but slowly used, absorption will be correspondingly slow; and all this adjustment of absorbing power may thus take place without any change in the permeability of the plastic membranes. It is probable that most cases of selective power are to be explained in this way. Just as there is a great difference in the use of the various absorbed solutes by different plants, so also there must be a corresponding difference in the amounts absorbed. Thus, Demoussy,[1] using equivalent quantities of several salts, found

[1] E. DEMOUSSY, "Absorption élective de quelques éléments minéraux par les plants," *Compt. rend.*, Vol. CXXVII (1900), pp. 970-73; *cf.* P. BOURGET, "Sur l'absorption de l'iode par les végétaux," *ibid.*, Vol. CXXIX (1899), pp. 768-70; IDEM, same title, *Bull. Soc. chim. de Paris*, Ser. III, Vol. XXIII (1899), pp. 40, 41.

that KNO_3 was taken out of a mixed solution two to six times as fast as $NaNO_3$ or $Ca(NO_3)_2$. He also observed that wheat plants absorb K ions two to three times as fast as those of Ca in equivalent solution. On the other hand, maize absorbs somewhat more Ca than K. Pfeffer[1] performed a series of experiments with fungi, which gave the general result, that the plant takes out of a mixed solution those solutes which are the best food for it. These would naturally be the ones whose diffusion tension would be first to diminish in the active cells.

There may be substances, however, such as certain poisons,[2] which react upon the membranes in such a way as to cause them to become impermeable. The membranes must, of course, be more or less permeable to such substances at first, else they could not react upon them. But such cases are very rarely met with. The so-called selective power is thus probably active usually, not in the absorbing organs, but in the cells where the substances are used in metabolism.

III. TRANSMISSION OF SOLUTES

An internal atmosphere exists in the plant, occupying the intercellular spaces, which are in communication throughout its body and which connect with the lenticels of the bark and with the air chambers and stomata wherever these occur. By means of this internal atmosphere, gaseous oxygen may reach the more deeply lying parts of the body, and the gaseous product of respiration in such parts may find its way to the surface. Thus, it is probable that the comparatively slow process of hydro-diffusion of gases is replaced by the much more rapid gas diffusion wherever the internal atmos-

[1] W. PFEFFER, "Ueber Election organischer Nährstoffe," *Jahrb. f. wiss. Bot.*, Vol. XXVIII (1895), pp. 205–68.

[2] PULST has recently shown that, while copper ions are readily absorbed by Mucor, Aspergillus, and Botrytis, they are not taken in by Penicillium. See C. PULST, "Die Widerstandsfähigkeit einiger Schimmelpilze gegen Metallgifte," *Jahrb. f. wiss. Bot.*, Vol. XXXVII (1902), pp. 205–63.

phere makes this possible. Mechanical movements of the plant, by wind, etc., probably hasten this diffusion by creating currents.

Apart from this gaseous diffusion, all transmission of solutes, whether gaseous, solid, or liquid, must take place in the form of aqueous solution. Diffusion of solutes from cell to cell takes place in accordance with the principles of diffusion, the tendency being ever to equalize the diffusion tension of all solutes throughout the extent of the solution.

In order to emphasize the fact already referred to that there is no relation between the turgidity produced by one solute and the diffusion of another into or out of the same turgid cell, the following example may be taken: Suppose an artificial cell whose membrane is impermeable to dissolved sugar but permeable to K and NO_3 ions. Such a membrane may be made from copper ferrocyanid. Let this cell be filled with a solution of sugar and potassium nitrate so made up that the partial pressures of the two substances are equal to each other, say five atmospheres. The total pressure of the solution is then ten atmospheres. Now let this cell be placed in distilled water. Since the membrane is permeable to KNO_3, this salt will immediately begin to diffuse outward, and diffusion will continue until its diffusion tension is practically as great outside as it is within the cell. No osmotic pressure will be manifested by the KNO_3, excepting the small amount due to friction in the membrane. But the sugar cannot pass out of the cell, and must therefore exert its full pressure upon the walls, making the cell turgid and manifesting a stretching force of nearly five atmospheres. Of course, as the cell becomes turgid a comparatively small amount of water will enter from without, and thus dilute the internal solution of sugar.

So far the illustration shows that it is possible for turgidity to be maintained while a substance is diffusing out of the

cell. This is just what probably occurs during the transmission of substances from one cell to another by diffusion. After a time, however, the artificial cell will come into equilibrium. The diffusion tension of the sugar is then just equaled by the resilience of the walls, and that of the inclosed KNO_3 by the diffusion tension of the same salt in the surrounding medium. No further changes of concentration will occur until some alteration is made in the conditions. Now let a few crystals of potassium nitrate be added to the external solution. They dissolve immediately and diffuse equally as far as the solution extends. But now the diffusion tension of this salt has been raised in the surrounding medium while it remains the same within the cell. However, since the membrane is permeable to KNO_3, this condition cannot last long; inward diffusion of K and NO_3 ions will soon equalize the tension within and without. Thus it is shown that a solute may diffuse not only *out of* but also *into* a cell, the latter remaining turgid meanwhile, through the action of another solute to which the osmotic membrane is impermeable.

Mass movements of the sap in stems, caused by changes in temperature, mechanical bending (as by the wind), etc., may aid very much in keeping the various solutes equally distributed throughout the inclosed solution. The mass movement occasioned in the solutions by evaporation from above (possibly also by sap pressure) must also aid in this. Within the cells the streaming movements of the protoplasm must act in the same manner, and the protoplasmic connections between adjacent cells probably sometimes set up mass currents which aid in the transmission of solutes from one cell to another. The latter consideration is probably of relatively great importance in the case of the transmission of carbohydrates and other plastic materials through the phloem region of stems. But by far the most impor-

tant factor in the distribution of solutes throughout the plant body, whether this be the plasmodial mass of a Myxomycete or the great body of a pine tree, is probably simple diffusion.

If it were not for the phenomenon of turgidity, the plasmic membrane would not be in condition to allow diffusion to take place readily. But the membranes do not directly aid in the transmission of solutes; they only hinder it.

CHAPTER IV

THE INFLUENCE OF THE OSMOTIC PRESSURE OF THE SURROUNDING MEDIUM UPON ORGANISMS

I. INTRODUCTORY

Although many researches have been carried out to determine what may be the influence upon the organism of the medium in which it is grown, it is only within the last few years that osmotic pressure has been investigated in this regard. Most experimenters have varied the chemical nature of the medium in which plants and animals were grown, and have argued from their experiments that the presence or absence of certain chemicals brings about certain effects within the organism. But if osmotic pressure can have any effect upon the behavior of the living being—and sufficient evidence has now been accumulated to show that it does have a very marked effect—then the results of all such researches must be considered as very questionable. Nearly all the published accounts of the influence of nutrient salts upon growth, reproduction, etc., in plants are subject to this criticism, that, while the author supposed he was varying a single factor, he was in reality varying at least two. Of course, any conclusions reached from research of this kind are not to be relied upon.

There are always two ways in which a nutrient fluid may affect the organisms placed in it, and these two ways correspond to the two entirely different sets of properties which are possessed by every solution. The solution may affect the animal or plant chemically, on account of its chemical properties, or it may have a physical effect, on account of its physical properties. Of course, since both sets of prop-

erties coexist in the same solution, it is possible and probable that the organism may often be affected in both ways at the same time.

By the chemical properties of a solution are meant the chemical nature of the solute or solutes. It is to be expected that a solution of cuprous sulfate will affect organic beings differently from a solution of cane sugar or one of sulfuric acid; these solutions are chemically very different. By physical properties are meant such qualities as viscosity, transparency, surface tension, osmotic pressure, etc. The latter is the only one of these which it is necessary to consider here. This property of osmotic pressure has been shown to be of general importance to the living being grown in ordinary nutrient solutions, but it has long been neglected in experiments with such solutions. Experimenters with nutrient fluids have varied the chemical nature of their solutions without taking into account the fact that in so doing they were very probably varying the osmotic pressure also.

When these workers have dealt with very weak solutions only, it is evident that the error thus introduced is practically negligible; the osmotic pressure must be very slight in all cases. Thus Ōno[1] showed that various mineral salts which are usually considered as poisons have an accelerating effect upon the growth of certain fungi when the solutions are very dilute. In this case the osmotic pressure is of such a low order that it may be left out of account. But suppose a case of another sort. It is also well known that a stronger solution of such a salt as cuprous sulfate will produce almost instant death. Shall it be argued, then, that life or death depends upon the number of Cu and SO_4 ions which may penetrate the living cells? Or shall it be

[1] N. Ōno, "Ueber die Wachsthumsbeschleunigung einiger Algen und Pilze durch chemische Reize," *Jour. Coll. Sci. Imp. Univ. Tōkyō*, Vol. XIII (1900), Part I: *Bot. Mag.*, Vol. XV (1900), p. 75; reviewed in *Bot. Gaz.*, Vol XXX (1900), p. 422.

argued that it is merely a question of osmotic pressure of the solution, and that the chemical nature of the cuprous sulfate has nothing to do with the response? A third possibility is to conclude that both these factors, always possessed in common by any solution, are active in bringing about the observed result. Obviously these two observations, that the organism lives in a weak solution of $CuSO_4$ and that it dies in a stronger one, are not sufficient to settle the question.

There are several different ways in which a plant cell may be affected by a solution into which it is plunged. If the solution be concentrated, it may have two effects: (1) Chemically, the solute may produce a response in the protoplasm by diffusing into it, and reacting with it in some way as yet not understood. Thus, the effect of a solution of $HgCl_2$ upon plant protoplasm is very different from that of cane sugar. (2) Physically, the solution may affect the cell by plasmolyzing it, or partially plasmolyzing it, or by reducing its active turgor pressure. It has been seen that this effect consists primarily in extracting water from the cell. Secondarily, it results in an increased concentration of the contained solution. This latter may again result in a chemical effect upon the living protoplasm, but of this we know absolutely nothing as yet. If the solution be a weak one, its physical effects will be just the reverse of those just mentioned, while its chemical effects will often be the same, but perhaps less marked. Physically, it will allow more water to diffuse into the cell, and there will result a rise in turgidity.

In order to answer the question stated above with regard to the nature of the effect produced by different concentrations of cuprous sulfate, experiments upon the same organism must be performed with other salts and with non-electrolytes, such as cane sugar, glucose, etc. In making these solutions

extreme care must be used to have them of exactly the same osmotic concentration as those of $CuSO_4$, which were previously used. Then, if the organism lives in all the dilute solutions, no matter of what substance, it may be concluded that the determining factor is one of osmotic pressure. If, on the other hand, the organism lives in the concentrated solutions of cane sugar, glucose, KNO_3, NaCl, etc., but dies even in a weak solution of $HgCl_2$ or $CuSO_4$, it must be concluded that the conditions of the medium which determine life or death are of a chemical nature. It is thus possible to analyze the effects of a solution by using a number of different solutes.

The primary effect upon an organism of an increase in the osmotic concentration of the surrounding medium is extraction of water, that is, it is a *drying* effect. The primary effect of a decrease in the osmotic concentration is the reverse, it adds water to the organism.

II. PRESENTATION OF MATERIAL

Following is a review of the several lines of experimental evidence which have been brought forward in connection with the question of the effect of variations in the concentration of the medium upon the living being. Since there is so little to be presented, the work upon both animals and plants will be included. The material at hand will be discussed under four heads: (1) The effect upon growth, (2) the effect upon reproduction, (3) the effect upon movement, and (4) the analogy between the effects of high osmotic pressure of the medium and those produced by other water-extracting processes.

a) Variations in the osmotic pressure of the surrounding medium: their influence upon the growth and form of organisms.—A number of observations upon various organisms have been made, all tending to the general conclusion

that growth takes place more slowly in a concentrated solution than in a weaker one. Loeb[1] found that the regeneration of decapitated tubularian hydroids occurs much more slowly in a concentrated than in a dilute solution. The optimum concentration lies considerably below the normal concentration of sea-water, in which these animals live naturally. Similar results were obtained by Yung,[2] working on tadpoles, and also by J. L. Frazeur (with annelids) and P. E. Sargent (with Dero vaga) in the laboratory of C. B. Davenport.[3]

The first of a very important series of observations dealing with the effect of external concentration upon cell division was made by Loeb[4] when he discovered the fact that, in fertilized Arbacia eggs which were placed in sea-water whose concentration had been raised by the addition of NaCl, the nuclei divided a number of times without the usual accompaniment of the segmentation of the entire egg. When these eggs with segmented nuclei were returned to normal sea-water, segmentation of the cytoplasm occurred suddenly, the number of segments corresponding, in general, to the number of parts into which the original nucleus had divided. Loeb concludes from these experiments that the extraction of water by high osmotic pressure causes a falling off in the irritability of the protoplasm. Whereas, a part of the normal process of cleavage, namely, that pertaining to the nucleus, is carried out, the remainder of it, segmentation of the egg, fails to occur in the strong solution. The cytoplasm fails to perform its part, although the nucleus is still

[1] J. LOEB, *Untersuchungen zur physiologischen Morphologie der Thiere.* II: *Organbildung und Wachsthum*, Würzburg, 1892.

[2] E. YUNG, "De l'influence des variations du milieu physico-chimique sur le développement des animaux," *Arch. des sci. phys. et nat.*, Vol. XIV (1885), pp. 502–22.

[3] C. B. DAVENPORT, *Experimental Morphology*, New York, 1899, p. 365.

[4] J. LOEB, "Ueber Kerntheilung ohne Zelltheilung," *Arch. f. Entwickl. d. Organismen*, Vol. II (1895), pp. 298–300.

active. Sperms which were placed in the strong solution lost their irritability, but regained it upon being returned to sea-water. Another instance which seems to show the partial loss of irritability by protoplasm from which water is osmotically extracted, is stated by the same author in the same article. Hearts of ascidians, crustaceans, and of embryo and adult vertebrates all beat less rapidly in strong solutions than in weak ones.

Morgan[1] repeated Loeb's experiments on Arbacia eggs with practically identical results. He made the added observation that the free nuclear division described above occurs in concentrated solutions, even though the eggs have not been fertilized. This author also gives valuable cytological notes on the nature of the free nuclear division.

This paper by Morgan has been followed by several others by Loeb,[2] the results of which may be brought together as follows: Unfertilized eggs of Arbacia, Strongylocentrotus, and Asterias, can all be made to develop parthenogenetically, if they are first placed for a time in sea-water, the concentration of which has been raised by addition of either an electrolyte (such as NaCl or $MgCl_2$) or a non-electrolyte (such as cane sugar or urea). They must then be returned to normal sea-water. In this artificial parthenogenesis development continues until the animal is in the Pluteus stage. This is as far as the development of normally fertilized embryos can be carried in aquaria. The author concludes that "there can be no doubt that the essen-

[1] T. H. MORGAN, "The Effect of Salt Solutions on Unfertilized Eggs of Arbacia," *Science*, N. S., Vol. VII (1898), p. 222.

[2] J. LOEB, "On the Nature of the Process of Fertilization and the Artificial Production of Normal Larvæ (Plutei) from the Unfertilized Eggs of the Sea Urchin," *Am. Jour. Physiol.*, Vol. III (1899), pp. 135–8; IDEM, "On the Artificial Production of Normal Larvæ from the Eggs of the Sea Urchin (Arbacia)," *ibid.*, Vol. III (1900), pp. 434–71; IDEM, "On Artificial Parthenogenesis in Sea Urchins," *Science*, N. S., Vol. XI (1900), pp. 612–14; IDEM, "Further Notes on Artificial Parthenogenesis and the Nature of the Process of Fertilization," *Am. Jour. Physiol.*, Vol. IV (1900), pp. 178–84; IDEM, "Artificial Parthenogenesis in Annelids (Chætopterus)," *Science*, N. S., Vol. XII (1900), p. 170.

tial feature in this increase in the osmotic pressure of the surrounding solution is a loss of water on the part of the egg."

Furthermore, the same author showed that a similar artificial parthenogenesis may be brought about in the case of Chætopterus, a marine annelid. Here another method of treatment would also bring it about, namely, a slight increase in the amount of potassium in the medium without any increase in its concentration. This is termed by Loeb *chemical fertilization* in contrast with *physical fertilization*, the form described above. Chemical fertilization by potassium is, so far, impossible in the eggs of Echinoderms. Very recently Loeb and Neilson[1] have shown that chemical fertilization by means of hydrogen ions is possible with eggs of Asterias, and the same sort of fertilization, but with calciumions, was brought about by Loeb and Fischer[1] in the case of eggs of the marine annelid, Amphitrite. These phenomena are interesting here mainly as they show that a chemical influence can bring about the same effect as extraction of water.

That plants grow less rapidly in concentrated solutions than in more dilute ones was first stated by Jarius,[2] who worked on the germination of seeds. With growing plants, Stange[3] showed that Pisum, Phaseolus, Lupinus, etc., increase in thickness more rapidly in concentrated solutions, while more rapid growth in length occurs in dilute ones. He suggests that the effect of the solution may be different upon the meristematic cells of the growing point and upon

[1] J. LOEB, M. FISCHER, AND H. NEILSON, "Weitere Versuche über künstliche Parthenogenese," Vorläufige Mittheilung, *Pflügers Arch. f. d. ges. Physiol.*, Vol. LXXXVII (1901), pp. 594–6.

[2] M. JARIUS, "Ueber d. Einwirkung von Salzlösungen auf d. Keimungsprocess d. Samen einiger einheimischer Culturgewächse," *Landwirtsch. Versuchs-Stat.*, Vol. XXXII (1886), pp. 149–78.

[3] B. STANGE, "Beziehungen zwischen Substratconcentration, Turgor und Wachstum bei einigen phanerogamen Pflanzen," *Bot. Zeitg.*, Vol. L (1892), p. 253.

those of the cambium. Vandervelde[1] experimented upon the germination of seeds which had been soaked twenty-four hours in various concentrations of several salts. As the solution became stronger the number of seeds to germinate decreased, but after a certain minimum of germination was reached, the number germinating again increased. This author suggests that the failure to germinate in the intermediate concentration is due to the penetration of the salts, while in stronger solutions little or no imbibition of water took place, and the seeds when planted were practically the same as when put into the solution. This subject has been recently taken up again by Buffum and Slosson.[2] These authors show that not only is imbibition of seeds greatly retarded by a concentrated solution (as was known before), but also germination and the growth of the plant are retarded in the same manner. This is true without regard to the chemical nature of the dissolved substance. Both electrolytes and non-conductors were used. Retardation of growth is not proportional to concentration, however, for an osmotic pressure of one hundred atmospheres retards growth only about twice as much as a pressure of ten atmospheres.

Regarding the maximum concentrations in which fungus growth can occur, investigations have been made by Eschenhagen and by Raciborski. Eschenhagen[3] found that this maximum was different for different fungi studied, but the concentration was about the same for different salts, seeming to show that it was a purely osmotic effect. For Penicillium the maximum concentration is about that of a five-normal

[1] A. J. J. VANDERVELDE, "Ueber den Einfluss des chemischen Reagentien und des Lichtes auf die Keimung der Samen," *Bot. Centralbl.*, Vol. LXIX (1897), pp. 337-42.

[2] E. E. SLOSSON AND B. C. BUFFUM, "Alkali Studies II," *Bulletin 39, Wyoming Agric. Exp. Sta.* (1898); B. C. BUFFUM, "Alkali Studies III," *Ninth Annual Report, Wyoming Agric. Exp. Sta.* (1899); E. E. SLOSSON, "Alkali Studies IV," *ibid.* (1899); B. C. BUFFUM AND E. E. SLOSSON, "Alkali Studies V," *Tenth Annual Report, Wyoming Agric. Exp. Sta.* (1900).

[3] F. ESCHENHAGEN, *Ueber den Einfluss von Lösungen verschiedener Koncentration auf das Wachsthum von Schimmelpilze*, Stolp, 1889.

cane-sugar solution, that is, about 111.5 atmospheres. Raciborski[1] found the maximum concentration for growth of Basidiobolus was that of a 6 per cent. solution of NaCl, or about seventeen atmospheres. Yasuda[2] published an account of some experiments upon infusoria which have a bearing here. He finds that these organisms are able to adjust themselves to solutions of quite high concentration, and that, in general, the limit of their power of adjustment seems to be at about the same osmotic pressure, no matter what salts are used. In other words, the limit to adjustment is apparently an osmotic one and depends upon withdrawal of water.

The experiments of the present author[3] upon the physiology of polymorphism in Stigeoclonium need to be considered here. In the stronger solutions (pressure from 323.7 cm. to 647.4 cm. Hg.) this alga takes the form of groups of spherical cells with somewhat gelatinous walls. Multiplication takes place rather slowly, cell division occurring in all directions and the daughter-cells immediately rounding up so far as they are not hindered by adjacent cells. In weak solutions (pressure below 161.8 cm. Hg.) the behavior is entirely different. The daughter-cells elongate into branching filaments composed of cylindrical cells and having the typical appearance of Stigeoclonium. Growth is much more rapid here than in the strong solutions. If filaments are transferred to a strong solution, the cells round up and break apart, thus producing the other form.

[1] M. RACIBORSKI, "Ueber den Einfluss äusserer Bedingungen auf die Wachsthumsweise des Basidiobolus ranarum," *Flora*, Vol. LXXXII (1896), pp. 107–32.

[2] ATSUSHI YASUDA, "Studien über die Anpassungsfähigkeit einiger Infusorien an concentrirte Lösungen," *Jour. Coll. Sci. Imp. Univ. Tōkyō*, Vol. XIII (1900), pp. 101–40. Reviewed in *Bot. Gaz.*, Vol. XXX (1900), p. 285.

[3] B. E. LIVINGSTON, (1) "On the Nature of the Stimulus Which Causes the Change of Form in Polymorphic Green Algæ," *Bot. Gaz.*, Vol. XXX (1900), pp. 289–317; IDEM, (2) "Further Notes on the Physiology of Polymorphism in Green Algæ," *ibid.*, Vol. XXXII (1901), pp. 292–302. Some parts of the discussion here given are quoted from these articles.

In the first series of cultures several modifications of Knop's solution were used. This solution consists of: $Ca(NO_3)_2$, four parts; $MgSO_4$, KNO_3, and K_2HPO_4, each one part, with the addition of a trace of iron. In order to determine whether a change in the concentration of this solution would affect the plant in a chemical or physical way, four modified solutions were made up, each being deficient in one of the four constituent salts. The deficient salt was reduced to one-tenth its normal quantity, and, the decrease in osmotic pressure thus brought about having been calculated, a sufficient amount of each of the three other salts was added to increase the pressure by an amount equal to one-third of the calculated decrease. Thus were obtained four solutions, all of which had the same osmotic pressure, but each of which was deficient in one salt.

The calculations for the pressure corrections were made both by the now obsolete method of De Vries, and by assuming that, in the concentrations used, ionization was complete. Solutions made by both methods gave the same results upon the plant, and after a first trial the second method of calculation was exclusively used. During the summer of 1901 the pressure of nearly all these solutions was tested by the freezing-point method. A table of the results so obtained will be found in the second paper cited on this subject. The error introduced by the assumption of complete ionization was found to be too small to interfere with the accuracy of the results in any degree, the discrepancy between the real and the calculated pressures lying well within the limits of the threshold of stimulation for this alga.

The cultures showed that all four modified solutions, and the normal Knop's solution also, influence the plant in exactly the same manner. The form of the alga is always determined by the osmotic concentration of the medium, and is not affected by the varying proportions of the constituent salts.

In the second series of cultures, besides the normal and modified Knop's solutions, two non-electroytes, cane sugar and lactose, were used, and it was found that here also the concentration is the controlling factor in the response of the plant. It must be noted, however, that to prevent the formation of filaments a somewhat higher concentration is required of these sugars than of the inorganic salt. This is perhaps due to a more ready absorption of the sugars and consequent rise in internal concentration. Whatever may be the cause of this phenomenon, it seems to be in accord with that noted by van Rysselberghe,[1] namely, that cells of Tradescantia, etc., develop a greater turgidity in salt solutions than in those of sugar.

It is thus shown conclusively that the changes in the growth of this plant which result from changes in the concentration of the medium are entirely dependent upon its osmotic pressure. This means that they are dependent upon the amount of water contained within the cells, for the strong solutions extract water, while the weak ones allow it to be absorbed.

In a weak solution vegetative growth is very much more rapid than in a strong one. This may be due to the fact that in a strong solution the water content of the protoplasm is reduced in amount below the limit for optimum lability. When the plant grows fastest and best it is in the filamentous form. In the weak solution, where activity seems to be at a maximum, the ions of the electrolytes, which are essential for metabolism, are not plentiful. This may suggest how the cylindrical form of cell with its increased surface[2] may

[1] VAN RYSSELBERGHE, "Réaction osmotique des cellules végétales à la concentration du milieu," *Mem. cour.*, pub. par l'Acad. roy. de Belg., Vol. LVIII (1898), pp. 1–101.

[2] In a cylinder, the lateral surface is greater than that of a sphere of the same volume, as long as the ratio of the length to the diameter equals or exceeds 2.727. In typical filament cells of this alga, the ratio of the diameters is 3, and it is often 4 and even greater. It is seldom less than 2.8. Thus, it is shown that the filament cell offers more surface to the surrounding medium through its lateral walls alone than does the palmella cell of equal volume.

be advantageous. At any rate, we may be sure that the greater surface of the cylinder puts the plant into better condition for exchange of material with its surrounding medium. On the other hand, the more concentrated solution not only withholds water from the cells, but presents a demand upon them for water. The cell meets this in part by offering as small a surface as possible to the solution. In this case, although the requisite ions may be present, and even in the right number, the scarcity of water in the protoplasm may so decrease the lability that rapid growth is impossible. We shall see that there is also a corresponding falling off in the reproductive activity in strong solutions. Perhaps this response is attributable to the increased general activity in weak solutions. It has no relation to the form of the cell, since zoöspores are produced from both spherical and cylindrical cells, as well as from those of intermediate shape.

It is to be emphasized that in the stronger solutions cell division and growth are not only retarded, but the direction of the dividing planes is curiously changed. Whereas in the weak solutions the cylindrical cells divide only by walls in one direction, the spherical cells of cultures in the more concentrated solutions divide in all directions. Whether this is due to the change in form of the cell, or directly to the water content of the protoplasm, cannot yet be decided.

What may be the mechanics of the rounding up of cylindrical cells when placed in a concentrated solution is one of the most important problems suggested by this research. The fact that the dead cellulose membrane is almost entirely reshaped during this process, without being dissolved, renders it probable that the change in form is directly caused by some change in turgidity within the cell. In a rounding cell the membrane moves and changes its form, and, since it is entirely inert, the source of this motion must be either in the activity of the protoplasmic body itself, or it must be in the

effective turgor pressure of the mass of liquid within. But since protoplasm and cellulose wall can be parted so readily during plasmolysis, the first alternative is well-nigh untenable. If the wall be forced into the spherical shape by a change in the pressure from within this must be brought about by a change in the volume of the contained liquids. Now, this slight change in volume which might produce a change in the turgidity of the cell is most probably due to an alteration in the amount of cell sap within the vacuole. When the surrounding medium suffers change in concentration, a change in the volume of the vacuole may come about through the protoplasmic sac either secreting liquid or acting merely as a semi-permeable membrane.

When filaments are placed in a concentrated solution their behavior suggests at once partial plasmolysis. Water may be extracted, the effective turgor pressure on the walls may be decreased, and by the forces of surface tension and cohesion the protoplasm may tend to round itself up into a sphere. If this be true, we have an explanation of the lateral bulging which accompanies the longitudinal shrinking of the cellulose envelope. If the protoplasm tended to assume a spherical form within the cylindrical wall, the pressure upon this would be decreased first at the angles. At the same time, it would be relatively increased upon the lateral walls near their middle. Thus would come about a bulging of the lateral walls outward, and hence a shortening of the cell and a drawing of the end walls toward each other. But the internal pressure is to be counted as almost nothing at the angles, while it is still considerable in the middle of each end wall. So the margins of the end walls would approach the middle of the cell more rapidly than do their central portions, and splitting of the common membrane of two adjacent cells would necessarily ensue. Several facts were observed in the cultures which seem to support some such hypothesis as the

one just stated. I have placed filaments in a solution where they were completely plasmolyzed and killed, without any change in form. In solutions a little less concentrated they are not plasmolyzed, but round up rapidly and soon die, often in the palmella condition. With a still lower pressure the filament cells round up more slowly and live. Another fact suggesting this idea is that floating filaments can resist a stronger solution, and can resist it longer, than sunken ones. The former are to some extent in contact with the air, and thus present less surface than the latter to the liquid. Still another observation bearing upon this hypothesis of partial plasmolysis is that cylindrical cells are the only ones which are able to change their form after they have become mature. A spherical cell must remain so till it divides, even if it be in a solution of very low pressure.

Raciborski[1] made what must be regarded as essentially the same observation as the one just discussed upon Basidiobolus, concerning the rounding up of cells and the change in direction of cross walls. He states that in strong solutions the cells became rounded and separated from one another, and that walls formed in all directions. Although he paid little attention to osmotic phenomena, yet it can hardly be doubted that Basidiobolus ranarum behaves in much the same way as does Stigeoclonium.

In a recent paper Beauverie[2] has described some interesting effects of the osmotic concentration of the medium upon fungi and higher plants. The concentration of his nutrient fluids was raised by the addition of NaCl—a very questionable method, especially in view of the proof brought forward by True[3] that this salt sometimes has a poisonous action.

[1] M. RACIBORSKI, "Ueber d. Einfluss äusserer Bedingungen auf d. Wachsthumsweise des Basidiobolus ranarum," *Flora*, Vol. LXXXII (1896), pp. 107–32.

[2] J. BEAUVERIE, "Influence de la pression osmotique du milieu sur la forme et la structure des végétaux," *Compt. rend.*, Vol. CXXXII (1901), pp. 226–29.

[3] R. H. TRUE, "The Physiological Action of Certain Plasmolyzing Agents," *Bot. Gaz.*, Vol. XXVI (1898), pp. 407–16.

Beauverie found that fungus hyphæ which normally grow upon the surface of the nutrient fluid, and even rise into the air, lose this habit in concentrated solutions, and remain, for the most part, submerged. Growth continues in these cases, but seems not to be as marked as in weak solutions. Of the hyphæ which do rise into the air from the concentrated medium, the cells are much shorter and broader than those which rise from a weak solution. Details are not given in the published account, but apparently we have here a very similar response to the one which was obtained in the case of Stigeoclonium.

The same author has also experimented upon flowering plants, *e. g.*, Pisum and Phaseolus. Grown in a strong solution, the stems of these plants are short and thick, and the roots show a remarkable development of cork tissue on their surfaces, with a slight development of pith. He also states that, while in weak solutions an upward bending of the roots normally occurs, in strong solutions these grow vertically downward. The upward bending in weak solutions has been ascribed heretofore to aërotropism. Perhaps the extraction of water which occurs in the strong solution changes the irritability of the roots so that they no longer respond normally to lack of oxygen. In the stems of Pisum and Phaseolus is perhaps presented another case of cells failing to elongate in a solution which extracts water. Much more experimentation is needed, however, before we can relate these responses in higher plants with those of algæ and fungi.

b) *The influence of external concentration upon reproduction.*—Raciborski[1] states that concentrated solutions check the formation of zygospores in Basidiobolus. In concentrated solutions Stigeoclonium[2] failed to produce any

[1] M. RACIBORSKI, "Ueber d. Einfluss äusserer Bedingungen auf d. Wachsthumsweise des Basidiobolus ranarum," *Flora*, Vol. LXXXII (1896), pp. 107–32.

[2] B. E. LIVINGSTON, (1) "On the Nature of the Stimulus Which Causes the Change of Form in Polymorphic Green Algæ," *Bot. Gaz.*, Vol. XXX (1900), pp. 289–317; IDEM, (2) "Further Notes on the Physiology of Polymorphism in Green Algæ," *ibid.*, Vol. XXXII (1901), pp. 292–302.

zoöspores, but these were formed in great numbers, and very rapidly, in the weak solutions. Since the formation of zoösporis is to be regarded as the result of protoplasmic activity, this fact is added evidence that the cosmotic extraction of water reduced the general activity of the protoplast.

c) *The influence of external concentration upon irritability.* (1) *Changes in irritability.*—That Loeb observed a loss of irritability in Echinoderm sperms when these were placed in a concentrated solution, and a return of it when they were brought back to normal sea-water, has already been noted. Richter[1] states that zoöspores of Tetraspora lose their activity in strong solutions, but regain it on being returned to normal sea-water. The writer found that zoöspores of Stigeoclonium lose their power of movement in concentrated solutions. Of interest here is also the observation of Engelmann[2] that the cilia of the epithelial cells which line the frog's œsophagus become much more active in pure water or a very weak solution than in a solution of the same concentration as the fluids of the animal's body.

Loeb[3] gives a very striking account of the reversal of a tropism by osmotic extraction of water. At ordinary temperatures the larvæ of Polygordius and certain Copepods are partly positively and partly negatively heliotropic. Above 25° C. they all react negatively, while below 10° C. the response is reversed, and they all become positively heliotropic. If NaCl is added to the normal sea-water in which these animals are living, they all react positively to light; if distilled water is added, they all react negatively.

[1] A. RICHTER, "Ueber die Anpassung der Süsswasseralgen an Kochsalzlösungen," *Flora*, Vol. L (1892), pp. 4–56.

[2] T. W. ENGELMANN, "Ueber die Flimmerbewegung," *Jena Zeitschr.*, Vol. IV (1868), pp. 321–479.

[3] J. LOEB, "Ueber künstliche Umwandlung positiv heliotropischer Thiere in negativ heliotropische und umgekehrt," *Pflügers Arch. f. d. ges. Physiol.*, Vol. LIV (1893), pp. 81–107; IDEM, *Physiology of the Brain*, New York, 1900, p. 198.

A similar reversal of tropism, in this case of geotaxis, was observed in Chromulina woroniniana by Massart.[1] Thus, by osmotically changing the amount of water in the protoplasm the irritability of these organisms can be reversed.

(2) *Osmotaxis.*—The concentration of the medium acts as a directing stimulus upon the motions of certain free-swimming organisms. This form of response has been named *osmotaxis*, in analogy to other similar responses to light, heat, chemicals, etc. An organism is said to be positively osmotactic when it swims from the weaker to the stronger solution where these are brought into contact. It is negatively osmotactic when it swims in the opposite direction.

Since the effect of high concentration of the medium is to extract water from the cell, it will be seen that there must be an identity of nature between this response and that of hydrotropism. An organism is positively hydrotropic when it bends away from a dryer and toward a moister atmosphere. This phenomenon is exhibited in roots, fungus sporophores, etc. It corresponds to negative osmotaxis, in which the organism swims from a region where water is extracted from its body to one where absorption can take place more freely. Since the conditions under which the two responses are made manifest are so very different, it is probably well to retain the word "osmotaxis."

Rothert[2] has recently devoted an article to the discussion of this subject. The following facts are mainly derived from this source, Stahl[3] showed that Myxomycete plasmodia, which had become accustomed to a certain concentration, would be repelled by any other concentration, either higher or lower. They are thus negatively osmotactic.

[1] J. MASSART, "La sensibilité à la concentration chez les êtres unicellulaires marins," *Bull. de l'acad. roy. de Belgique*, Ser. III, Vol. XXII (1891), pp. 148-67.

[2] W. ROTHERT, "Beobachtungen und Betrachtungen über taktische Reizerscheinungen," *Flora*, Vol. LXXXVIII (1901), pp. 371-421.

[3] E. STAHL, "Zur Biologie der Myxomyceten," *Bot. Zeitg.*, Vol. XLII (1884), pp. 145 ff.

Massart,[1] experimenting upon certain bacteria, found that they were negatively osmotactic to solutions of many different substances. The proof that the phenomenon is osmotaxis and not chemotaxis lies in the fact that the organisms were repelled always at the same osmotic concentration, irrespective of the chemical nature of the solute. Rothert found Treptomonas agilis positively osmotactic toward solutions which are so concentrated that they kill the organism by plasmolysis. Only such solutes are available for experiments upon osmotaxis as are known to be unable to penetrate the protoplasm of the organism to be tested. Of course, if penetration occurs, the difference in concentration within and without the cell can last but a short time; it will soon be equalized by the inward diffusion of the solute.

d) The analogy between the effects of high osmotic pressure of the medium and those produced by other water-extracting processes.—Attention has already been called to the fact that a lowering in temperature is often accompanied by giving out of water. Thus Spirogyra filaments when cooled in olive oil may be seen to give off water before freezing. It seems probable that in this case the protoplasm becomes more permeable at these low temperatures and thus the solute escapes with the solvent. If this is true, we cannot look upon cold plasmolysis as producing a concentration of the cell sap. It would only decrease its volume.

Of course, a part of the shrinkage in such a case could be accounted for by the diminution of osmotic pressure due to cooling. If the original internal pressure were p at t°C., then it would decrease to $p - \frac{t'p}{273 + t}$ at t'°C. The pressure of the external solution will decrease according to the

[1] J. MASSART, "La sensibilité à la concentration chez les êtres unicellulaires marins," *Bull. de l'acad. roy. de Belgique*, Ser. III, Vol. XXII (1891), pp. 148–67; IDEM, "Sensibilité et adaptation des organismes à la concentration des solutions salines," *Arch. de biol.*, Vol. IX (1899), pp. 515–70.

same principle, but since its original pressure, say s, was much smaller than p, its decrease for the same fall of temperature will not be so great as that of the internal solution. This may be shown thus:

$$\frac{t'p}{273+t} > \frac{t's}{273+t},$$

when

$$p > s.$$

Thus, the internal and external osmotic pressure will be more nearly the same at a low temperature than at a higher one. The two pressures should become equal at absolute zero.

No measurements have been made to determine whether the decrease in volume of the Spirogyra vacuole is proportional to the approach of the external and internal concentrations toward each other. This should not be a difficult thing to settle. But, as has already been stated (page 75), there is cryoscopic evidence that the extruded liquid is not pure water.

The identity of the responses obtained by Loeb with Copepods and Polygordius larvæ when these were subjected to cold and to high concentrations, has also been noted (page 139). A similar change of tropism occurs among those plant lice which exist in two forms, one winged and the other wingless. The growth of wings in the wingless form can be called forth either by low temperature or by allowing the plants upon which the animals are feeding to dry, thus depriving the latter of water. While in the wingless condition these lice are negatively heliotropic, but upon developing wings they become positively so. Here is a reversal of tropism brought about by withdrawal of water, but this experiment also shows that, although the general protoplasmic activity may be depressed by this treatment, yet certain special activities (*e. g.*, those involved in wing formation) may be accelerated.

It is generally known that lowering of the temperature of an animal heart causes the beating to become less rapid. This is perfectly parallel to the falling off in heart activity in strong solutions, as observed by Miss Shively and recorded by Loeb (page 129).

In my own experiments on Stigeoclonium,[1] it was found that the organism responds to drying on a porous plate in exactly the same way as it does to change from a weak to a strong solution.

Recently, Greeley[2] has shown that by cooling Stentor cœrulcus the same cessation of activity and rounding up was brought about as when the animals were subjected to the action of concentrated solutions. However, the effect of the solution was not reversible, for the animals could not be revived. The same author has shown that cold plasmolysis in Spirogyra is reversible, that a rise in temperature brings the plasmolyzed alga back to its normal condition.

During the summer of 1901 Greeley[3] was able to produce artificial parthenogenesis of Echinoderm eggs by merely keeping them for a time at a low temperature. In these cold-fertilized eggs, development went as far as in normally fertilized ones under artificial conditions.

In general, then, it may be concluded that there is a striking analogy between the responses obtained in these various organisms by treating them with strong solutions and by extracting water from them in any other way. How much further we may go in this, remains for future experiment to show.

[1] B. E. LIVINGSTON, "Further Notes on the Physiology of Polymorphism in Green Algæ," *Bot. Gaz.*, Vol. XXXII (1901), pp. 292–302.

[2] A. W. GREELEY, "On the Analogy between the Effects of Loss of Water and Lowering of Temperature," *Am. Jour. Physiol.*, Vol. VI (1901), pp. 122–8.

[3] A. W. GREELEY, "Artificial Parthenogenesis Produced by a Lowering of the Temperature," *Am. Jour. Physiol.*, Vol. VI (1902), pp. 296–304.

III. SUMMARY OF THE CHAPTER

As far as investigation has gone, it has been found that growth is accelerated in weak solutions and retarded in concentrated ones. The term "growth" here includes, not only enlargement, but also the process of cell division. Also, in some cases at least, the direction of new walls is profoundly influenced by the concentration of the surrounding medium. In general, all vital processes are retarded in concentrated solutions. Reproduction, being a peculiar form of cell division, appears in some cases to be entirely dependent upon the osmotic pressure of the surrounding medium. Irritability is also greatly influenced by external pressure. Not only is this function retarded in concentrated solutions, but in some forms the direction of response to a given stimulus may be reversed by a sudden change in the osmotic surroundings. The comparative concentration of the external and internal solutions acts, in many cases, as a stimulus upon the organism, giving rise to the phenomena of osmotaxis.

All the effects of high concentration of the surrounding liquid seem to be due to extraction of water from the living cells. They may be due either to a drying-out process or to decrease in turgidity. That they are sometimes due to the former is proved by curious analogies between the various processes which extract water from the protoplasm. Whether or not this extraction of water from the protoplasm itself is the direct cause of the responses to concentrated solutions, is not yet known. The effect may be a chemical one, due to the increased concentration of the contained solutions.

INDEX

Absorption: of gases, 115; of solids and liquids, 113; of solutes, 115.
Acids: influence of, on permeability, 61, 74; penetrating power of, 64.
Acetanilid, in plasmolysis, 63.
Acetone, in plasmolysis, 63.
Action of protoplasmic membrane, 80.
Alcohol, ethyl, in plasmolysis, 63.
Alcohols: penetrating power of, 71; aliphatic, in plasmolysis, 63.
Alkalies, penetrating power of, 64.
Ammonia, penetrating power of, 64.
Ammonium carbonate, 77.
Ammonium chlorid, ionization of, 23.
Amphitrite, eggs of, 130.
Amylase, penetrating power of, 71.
Anæsthetics, effect of, on permeability, 78.
Anilin, in plasmolysis, 63.
Anilin dyes, penetrating power of, 66.
Animal cells, permeability of, 64.
Antipyrin, penetrating power of, 64.
Apple, pressure of sap, 86.
Arbacia, eggs of, 128.
Arengo, exudation pressure of, 102.
Arrhenius, 18, 24.
Artari, 70.
Askenasy, 111.
Aspergillus: permeability of, 67; solutes of, 83.
Asterias, eggs of, 129, 130.
Asci, bursting of, 54.
Atmosphere, internal, 120.
Atomic theory, 3.
Avogadro, principle of, 11.
Bacteria, plasmolysis of, 58, 61.
Bacterium termo, plasmolysis of, 61.
Bases, influence of, on permeability, 61, 74.
Basidiobolus, in osmotic solutions, 132, 138.
Bean, permeability of, 65.
Beauverie, 137.
Beckmann, 37, 39.
Beet: permeability of, 62, 64, 78; solutes of, 83.
Begonia, permeability of, 62, 73, 76.
Berberis, pulvini of stamens in, 76.
Blackman, 116, 117.
Bleeding, 102; theory of, 104.
Blood corpuscles, 54, 57.
Blue, methyl, penetrating power of, 66, 77.
Böhm, 111.
Bonnier, 73, 98.
Bouilhac, 70.
Bourget, 69, 119.
Bower, 88.
Boyle, principle of, 10.
Browne and Escombe, 116.
Buffum and Slosson, 113.
Burgarszky, 18.
Bursting of cells, 54.
Cabbage, pressure of sap, 86.
Caffein: in plasmolysis, 63; penetrating power, 64, 77.
Calcium: absorption of, 67, 120; penetrating power of, 69.
Calcium nitrate, absorption of, 120.
Campbell, 66.
Caoutchouc, membrane of, 82.
Carbon dioxid: absorption of, 93, 115; from roots, 72; influence of, on transpiration, 113; penetrating power of, 70.
Carbonates, penetrating power of, 64.
Carrot, pressure of sap, 86.
Celery, pressure of sap, 86.
Cell wall, permeability of, 55.
Chaetomorpha, permeability of, 62.
Chaetopterus, chemical fertilization of, 130.
Chemical theory of semi-permeability, 82.
Chenopodiaceae, absorption of iodin by, 69.
Chlorids, penetrating power of, 78.
Chromulina, reversal of tropism in, 140.
Chloral hydrate, in plasmolysis, 63.
Cholesterin, 81.
Cilia, in osmotic solutions, 139.
Clausen, 116.
Cocos, exudation pressure of, 102.
Codium, permeability of, 78.
Coefficients, isosmotic, 56.
Cohnheim, 83.
Colloids, 27, 49.
Conductivity of saps, 85.
Copeland, 77, 83, 88, 105, 108, 111.
Copeland and Kahlenberg, 69.
Copepods, reversal of tropism in, 139.

COPPER, accumulation of, 69.
COPPER FERROCYANID MEMBRANE, 82, 112.
COPPER SULFATE, 110.
COUPIN, 68.
CRYSTALLOIDS, 49.
CURCUMA, permeability of, 62.
CURTIS, 54.
CURVATURE, rôle of turgidity in, 89.
CYNARA, 78.
CZAPEK, 72, 74.
DANDENO, 22, 68, 71, 72, 98.
DAVENPORT, 31, 128.
DEATH, theory of, 75.
DEMOUSSY, 67, 119.
DERO VAGA, regeneration of, 128.
DEVAUX, 69.
DE VRIES, 55, 56, 61, 62, 64, 65, 74, 77, 83, 84.
DIFFUSION, of gases, 9.
DIFFUSION TENSION, of solvent, 30.
DIGESTION, outside the body, 71, 72.
DIXON, 111, 112.
DUTROCHET, 111.
DYES, anilin, penetrating power of, 66.
ECTOPLAST, 51, 53, 80, 82.
EGGS, parthenogenesis of, by cold, 143.
ELECTROLYTES, in plasmolysis, 56.
ELODEA, permeability of, 66.
EMBRYO, permeability of, 72.
ENDOSPERM, permeability of, 72.
ENGELMANN, 139.
ENVIRONMENTAL FACTORS, 47.
ENZYMES, penetrating power of, 71, 72.
EPIDERMIS, permeability of, 117.
ESCHENHAGEN, 131.
ETHER, ethyl, in plasmolysis, 63.
ERRERA, 59.
EUPHORBIA, nectaries of, 79.
EVAPORATION, 110.
EXUDATION: nature of, 73; from wounds, 102; from glands, 71, 96.
EXUDATION PRESSURE, theory of, 104.
FAGOPYRUM, solutes of, 83.
FEHLING'S SOLUTION, 65.
FERRIC PYROLIGNATE, 110.
FERTILIZATION BY COLD, 143.
FICK, 18.
FILTER THEORY OF SEMIPERMEABILITY, 80.
FLUSIN, 82.
FORM, retention of, 87.
FORMALDEHYDE, 63.
FRAZEUR, 128.
FUCHS, 104.
FUCUS, permeability of, 74.
FUNGI, in osmotic solutions, 138.
FURFUROL, 63.
GASES: diffusion tension of, 9; mixed, 11; absorption of, 115; transmission of, 120.
GAY-LUSSAC, principle of, 10.
GLYCERIN, in plasmolysis, 56, 62, 63, 64, 67, 77, 79.
GLUCOSE: in turgor, 84; penetrating power of, 61, 65, 67, 70, 79.
GODLEWSKI, 108.
GOGORZA AND GONZALEZ, 54.
GRAHAM, 18.
GRAM MOLECULE, 20.
GREELEY, 75, 143.
GROWTH, rôle of turgidity in, 88.
GRYNS, 57.
GUNNERA, solutes of, 83.
GUTTATION, 73, 74, 98.
HAMBURGER, 57, 83.
HANSTEEN, 66.
HARTIG, 110.
HAUPT, 79, 100.
HEARTS: in osmotic solutions, 129; effect of cold upon, 143.
HEALD, 69, 85.
HEDIN, 57, 83.
HELIANTHUS: permeability of, 65, 74; solutes of, 83, 84.
HILBURG, 78.
HÖBER, 18, 83.
HONEY-DEW, 98.
IMBIBITION, 93.
INFUSORIA, in osmotic solutions, 132.
INTERNAL ATMOSPHERE, 120.
IODIN, penetrating power of, 68, 69.
IONS, in absorption, 53.
IONIZATION: of gases, 23; of solutes in liquids, 24.
IRRITABILITY, changes of, 139.
JARIUS, 130.
JANSE, 62, 65, 77, 108.
JENNINGS, 63.
JUMELLE, 112.
JUNG, 128.
KAHLENBERG, 22.
KINETIC THEORY OF MATTER, 4.
KLEBS, 62, 88.
KNOP'S NUTRIENT SOLUTION, 132.
KOHLRAUSCH, 42.
KOHLRAUSCH AND HOLBORN, 42, 43.
KÖPPE, 57.
KOSSAROFF, 113.
KÖVESI, 83.
KRABBE, 75.
KRAUS, C., 102.
KRAUS, G., 83.

LAURENT, 67, 71.
LEAVES: absorption by, 68, 103; exudation from, 99; guttation of, 74; permeability of, 71.
LECETHINS, 81.
LEHMAN, 69.
LEITZMANN, 116.
LEMNA, permeability of, 76.
LICE, reversal of tropism in, 141.
LIDFORSS, 54.
LIEBERMANN, 18.
LILIACEÆ, 69.
LIQUIDS: absorption of, 118; diffusion of, 12, 13.
LIVINGSTON, 132, 138, 143.
LÖB, 57.
LOEB, 53, 74, 128, 129, 139.
LOEB, FISCHER, AND NEILSON, 130.
LUPINUS: in osmotic solutions, 130; solutes of, 84.
MACDOUGAL, 69, 119.
MAQUENNE, 74, 84, 85.
MASSART, 61, 140, 141.
MATRUCHOT AND MOLLIARD, 70, 75.
MATTER: nature of, 3; states of, 6.
MAYER, 84.
MEDIUM, influence of, 124.
MEERBURG, 36, 82.
MEMBRANES: cellulose, 51; copper ferrocyanid, 82.
MEMBRANES: protoplasmic, 49; action of, 80.
MERCURIC CHLORID: effect on permeability, 74; penetrating power, 68.
METHYL BLUE, penetrating power of, 66, 77.
METHYL CYANID, in plasmolysis, 63.
METHYL VIOLET, penetrating power of, 66.
MIMOSA, pulvini of, 77.
MOHL, 98.
MOLDS, bursting of, 54.
MOLISCH, 65, 72, 75, 102, 103, 106.
MORGAN, 129.
MORSE AND HORN, 36.
MUCOR, exudation from, 99.
MÜLLER, 116.
MUSCLE, permeability of, 74.
MYRIOTONIE, 59.
NACCARI, 18.
NATHANSOHN, 78.
NECTARIES: artificial, 99; conditions for secretion of, 101; theory of, 96.
NERNST, 18.
NERNST-PALMER, 37, 38, 40.
NITRATES, test for, 65.
NOLL, 54.
NOSTOC, permeability of, 70.
NUCLEI: frozen, dried, etc., 76; in osmotic solutions, 128.
OLTMANNS, 74, 102.
ONION: permeability of, 78; solutes of, 83.
ONO, 69, 125.
OSMOTAXIS, 140.
OSMOTIC PRESSURE: in general, 28; demonstration of, 32; of electrolytes, 27; of non-electrolytes, 25; indirect measurement, by freezing-point, 37; by boiling-point, 38; by vapor tension, 39; calculation of, for electrolytes, 42; for non-electrolytes, 41; compared to drying, 141.
OSTWALD, 42.
OSTWALD-WALKER, 16, 40, 42.
OVERTON, 63, 71, 81.
OXYGEN: absorption of, 115; effect of, on permeability, 78; penetrating power of, 70.
PARAMŒCIA, permeability of, 63.
PARTHENOGENESIS: 129; by cold, 143.
PEAR, pressure of sap of, 86.
PEAS, solutes of, 84.
PENICILLIUM, in osmotic solutions, 131.
PERMEABILITY OF PROTOPLASM: 60, 64, 67, 68, 69, 70, 118; outward, 63, 71; variations in, 72; effect of on turgidity, 86.
PFEFFER, 35, 50, 55, 64, 66, 68, 77, 78, 104, 105, 120.
PHASEOLUS: in osmotic solutions, 130, 138; permeability of, 65, 78; solutes of, 83.
PHENOL, in plasmolysis, 63.
PHLOROGLUCIN, in plasmolysis, 63.
PHOSPHORIC ACID, from roots, 72.
PHOTOSYNTHESIS, 117.
PHYTOLOCCA, penetrating power of sap of, 98.
PICRIC ACID, 110.
PILOBOLUS, bursting of, 54.
PISUM: in osmotic solutions, 130, 138; solutes of, 83, 84.
PITRA, 103.
PLANT LICE, reversal of tropism in, 142.
PLASMOLYSIS: in general, 54, 60; by cold, 75; of bacteria, 58; effect of on growth, 88; on permeability, 74.
PLATINUM CHLORID, 65.
POISONS, permeating power of, 68.
POLLEN GRAINS, bursting of, 54.
POLYGORDIUS, reversal of tropism in, 139.
POLIMORPHISM: in Basidiobolus, 137; in fungi, 138; in Stigeoclonium, 132.
POTASSIUM: absorption of, 67, 120; penetrating power of, 69.
POTASSIUM CHLORID, in turgor, 83.

POTASSIUM NITRATE: absorption of, 120; in plasmolysis, 56, 61; in turgor, 83; penetrating power of, 65, 68, 74, 77.
PRESSURE: exudation, 102; gas, 9; osmotic (see Osmotic pressure).
PROTOPLASM, 49.
PROTOPLASMIC MEMBRANES, action of, 80.
PURIEWITCH, 78.
QUERCUS, copper in, 69.
QUINCKE, 82.
RACIBORSKI, 132, 137, 138.
REINHARDT, 88.
REPRODUCTION, in osmotic solutions, 138.
RICHARDS, 68.
RICHTER, 139.
RISE OF WATER, 107.
ROOT HAIRS, exudation from, 75.
ROOTS, permeability of, 69.
ROTHERT, 140.
SALTS, inorganic, penetrating power of, 67, 71.
SAMBUCUS, permeability of, 76.
SAP: cell, 52; expressed, conductivity of, 85.
SARGENT, 128.
SCHEFFER, 18.
SCHNEIDER, 112.
SCHWENDENER, 76.
SEEDS, in osmotic solutions, 130, 131.
SELECTIVE POWER, 118.
SIPHONEÆ, bursting of, 54.
SKERTSCHLEY, 69.
SLOSSON, 131.
SODIUM, penetrating power of, 69.
SODIUM CHLORID: in plasmolysis, 61; penetrating power of, 68, 74; in medium, 137.
SODIUM NITRATE: absorption of, 120; penetrating power of, 79.
SOLIDS, absorption of, 118; diffusion of, 14.
SOLANUM, absorption by, 69.
SOLUTES: absorption of, 115; active, nature of, 83; transmission of, 120.
SOLUTION, Knop's, 132.
SOLUTIONS: defined, 16; properties of, 124; molecular, 21; normal, 21; of gases in liquids, 17; of liquids in liquids, 16; of solids in liquids, 18; terminology for, 20.
SOLUTION THEORY OF SEMIPERMEABILITY, 80.
SPERMS, in osmotic solutions, 129, 139.
SPIROGYRA: cold plasmolysis of, 75, 141; permeability of, 62, 65. 68.
STAHL, 140.
STANGE, 84, 130.
STENTOR, cold plasmolysis of, 75, 143.
STEVENS, 69.
STICHOCOCCUS, permeability of, 70.
STIGEOCLONIUM: drying out of, 143; in osmotic solutions, 132, 138; zoospores in solutions, 139.
STOMATA, 93.
STRASBURGER, 110.
STRATIOTES, permeability of, 62.
STRONGYLOCENTUTUS, eggs of, 129.
SUCRASE, penetrating power of, 71.
SUGAR, CANE: in plasmolysis, 61; penetrating power of, 65.
SUNFLOWER, permeabilty of, 65.
SUPPORT, mechanical, by turgidity, 87.
SURROUNDING MEDIUM, influence of, 124.
SUTHERST, 85.
TAMMAN, 36.
TEMPERATURE: influence of, on absorption, 95; influence of, on permeabilty, 75; influence of, on turgor, 77.
TENSION, diffusion, of solvent, 30.
THEORIES: of matter, 3; of semipermeability, 82.
THUNBERGIA, permeability of, 71.
THUYA, transpiration of, 111.
TONIE, 59.
TONOPLAST, 52, 53, 80, 82.
TRADESCANTIA, permeability of, 56, 62, 76.
TRANSMISSION: of solutes, 115, 120; of water, 95.
TRANSPIRATION, 93, 96.
TRANSPIRATION STREAM, 107.
TRAUBE, 50.
TROPISM, reversal of, 139.
TRUE, 68, 137.
TURGESCENCE, 52.
TURGIDITY: nature of, 49, 52, 53; maintenance of, 86, 121; relation of, to activity, 87.
TURGOR, 52, 55, 74.
TURNIP, pressure of sap of, 85.
UREA: in plasmolysis, 62; penetrating power of, 77, 79.
VACUOLE, 52.
VANDERVELDE, 131.
VAN RYSSELBERGHE, 76, 134.
VAN'T HOFF, 25, 37, 40.
VEGETABLE MARROW, pressure of sap of, 85.
VESQUE, 95.
VICIA: nectaries of, 79; roots of, 65.
VIOLET, methyl, penetrating power of, 66.
VOIGTLANDER, 18.
VON MAYENBURG, 67, 83, 118.
WALDEN, 36.

WALKER, 40.
WALL, cell, 51. 52.
WATER: absorption of, by plant cells, 91; absorption of, by muscle, 53: in cellulose wall, 52; loss of, 95; transmission of, 95.
WATER PORES, 74, 96.
WESTERMEIER, 108.
WHETHAM, 43.
WIELER, 65, 102, 108.
WIESNER AND MOLISCH, 116.
WILSON, 98.
WLADIMIROFF, 58.
WOODS, 112.
WORTMANN, 66.
YASUDA, 132.
ZEA, solutes of, 83.
ZOOSPORES, in osmotic solutions, 139.

DATE DUE

APR 17 00

4/04

CLEAN

4/9/04

Clean 11/2/05

QH
611
.L78

9830

Livingston

The rôle of diffusion and osmotic pressure in plants

AUSTIN COLLEGE LIBRARY

ABELL LIBRARY - AUSTIN COLLEGE
AM 000 137 787

06-CPH-783